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VON

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Aus dem Max-Planck-Institut für Biologie, Tübingen

ÜBER DIE KOMBINATION VON *WA* (WEISSÄUGIG) UND *BIOCHEMICA* BEI *EPHESTIA KÜHNIELLA*

Von

ALFRED KÜHN und BRIGITTE BERG

Mit 3 Textabbildungen

(Eingegangen am 22. September 1955)

Bei der Mehlmotte sind drei Mutationen bekannt, die auf die Bildung der Ommochrome und auf die der fluoreszierenden Stoffe einwirken: *a*, *wa* und *bch*. Das mutierte Allel *a* vermindert die Ommochrombildung und steigert die Bildung der fluoreszierenden Stoffe, unter denen Pteridine die Hauptrolle spielen; *wa* unterdrückt die Ommochrombildung und gleichzeitig auch die der fluoreszierenden Stoffe im Auge (HADORN und KÜHN 1953). Die Allele a^+ bzw. *a* wirken also auf die beiden Stoffgruppen gegensinnig, *wa* wirkt gleichsinnig radikal hemmend. Durch *bch* wird das gelbe Pigment der Corneapigmentzellen beseitigt, und das Pigment der Retinula- und Nebenpigmentzellen wird in seinem Farbton verändert; gleichzeitig verschwinden im Fluoreszenzmuster alle Flecken außer zwei Pteridinen, deren Substanz gegenüber der Wildform und der Mutante *a* stark vermehrt ist. Also auch *bch* zeigt eine Wirkung auf Ommochrompigmente und Pteridine.

Tabelle 1. F_2 *aus der Kreuzung weißäugig* × *biochemica.* n = 1725 aus 12 Zuchten

	$\frac{+}{\cdot}\ \frac{+}{\cdot}$	$\frac{+}{\cdot}\ \frac{bch}{bch}$	$\frac{wa}{wa}\ \frac{+}{\cdot}$	$\frac{wa}{wa}\ \frac{bch}{bch}$
Befund	1193	252	220	60
Spaltzahl	11,06	2,34	2,04	0,56
Erwartung ± 3 m .	9 ± 0,57	3 ± 0,45		1 ± 0,29
Abweichung in % .	+23	−22	−32	−44

In einer früheren Untersuchung (KÜHN und BERG 1955) wurde die Kombination von *bch* mit *a* geprüft. Dabei erwies sich *a* in bezug auf die durch Ommochrome bedingte Augenfarbe epistatisch, aber den fluoreszierenden Stoffen im Auge gegenüber als hypostatisch: Die Augen sind rot bzw. durch die Anwesenheit aufhellender Modifikatoren orangefarbig; aber anstatt des für *a* sonst charakteristischen reichen Musters fluoreszierender Stoffe sind nur die 2 Pteridine vorhanden, die *bch* entsprechen, und deren Substanz ist wie bei *bch* vermehrt (KÜHN und BERG 1955).

Hier wird über Kreuzungen berichtet, welche über das *Zusammenwirken von wa mit bch* Aufschluß geben sollen.

In der F_2 aus *weißäugig* × *biochemica* spalten Dunkeläugige und Weißäugige heraus. Beide Gruppen werden durch die Chromatogramme gegliedert einerseits in +/·; +/· und +/·; *bch*/*bch*, andererseits *wa*/*wa*; +/· und *wa*/*wa*; *bch*/*bch*, in denen sich *bch* in später zu besprechender Weise geltend macht. Tabelle 1 zeigt die *Spaltverhältnisse*. Bei den homozygoten doppelt Rezessiven fehlt fast die Hälfte der Erwarteten, bei den Tieren mit je einem homozygoten rezessiven Allel ist ein geringerer Ausfall ebenfalls gesichert. Bei diesen 3 Gruppen ist also die Vitalität gegenüber den Dominanten gesenkt. Da *bch*/*bch* an sich gegenüber

+/· nicht benachteiligt ist (KÜHN und BERG 1955), müssen auch heterozygote Kombinationen von *wa* mit *bch* schwächend wirken.

In der *Entwicklungsgeschwindigkeit* eilen die Individuen mit mindestens einem +-Allel in beiden Genpaaren den übrigen Genotypen voraus (Abb. 1). Die am stärksten in der Vitalität herabgesetzten *wa/wa*; *bch/bch* zeigen die größte Streuung und die stärkste Entwicklungsverzögerung, wenn der Unterschied von den beiden anderen vitalitätsschwachen Gruppen auch nicht statistisch gesichert ist.

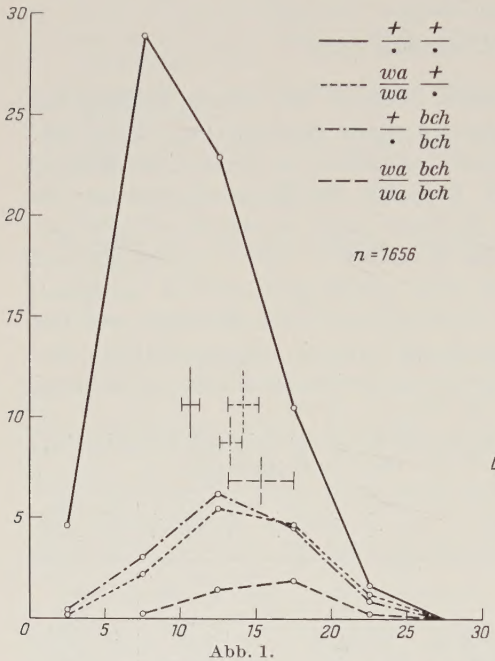

Abb. 1.

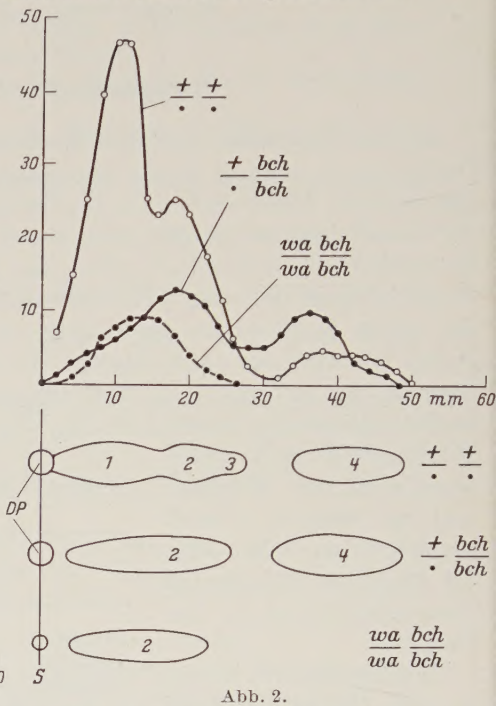

Abb. 2.

Abb. 1. Variationskurven der Entwicklungsgeschwindigkeiten der herausspaltenden Genotypengruppen. Abszisse: Tage nach dem Beginn des Schlüpfens aus der Puppe. Ordinate: Prozent Tiere; Mittelwerte der Kurve und Spielräume von 3 m

Abb. 2. Fluorometerkurven (Apparate und Meßverfahren: KÜHN 1955) der Augenstoffe der Genotypen; Abszisse: Folge der gemessenen, jeweils 2 mm breiten Abschnitte der Chromatogramme. Ordinate: Galvanometerwerte. Unten Umrisse der Flecke der Chromatogramme der Genotypengruppen. Farben der Flecke: *1* eisgrün, *2* ultramarinblau, *3* grün, *4* kobaltblau; *DP* dunkles Pigment, *S* Startlinie

Die *Weißäugigen mit bch/bch* sind daran kenntlich, daß in den Chromatogrammen ein blauer Fleck vorhanden ist. Er entspricht dem unteren ultramarinblauen Fleck von *biochemica* und besteht aus Isoxanthopterin (s. die Farbaufnahmen bei KÜHN und BERG 1955, Abb. 7e).

In Abb. 2 sind die *Fluorometerwerte* von Papierchromatogrammen von F_2-Individuen der Genotypen +/·; *bch/bch* und *wa/wa*; *bch/bch* mit der Kurve von +/·; +/· zusammengestellt. Darunter sind die Umrisse der Flecken der Fluoreszenzmuster gezeichnet.

Die Meßwerte der einzelnen Individuen variieren bei allen 3 Gruppen (Abb. 3). Bei den Weißäugigen *wa/wa*; *bch/bch* kann der blaue Fleck ziemlich groß und weit ausgezogen sein und in der Meßkurve auch ein kleines, dem zweiten Fleck von *bch* entsprechendes Maximum zeigen (Abb. 3i), oder er kann sehr klein sein und nur eine kurze und niedere Meßkurve ergeben (Abb. 3m).

In bezug auf die Augenpigmentierung ist also wa vollkommen epistatisch, in der Bildung von Pteridinen setzt sich aber bch in der für dieses Gen charakteristischen Weise durch, wenn seine Epistase auch nicht so vollständig ist wie gegenüber *a*.

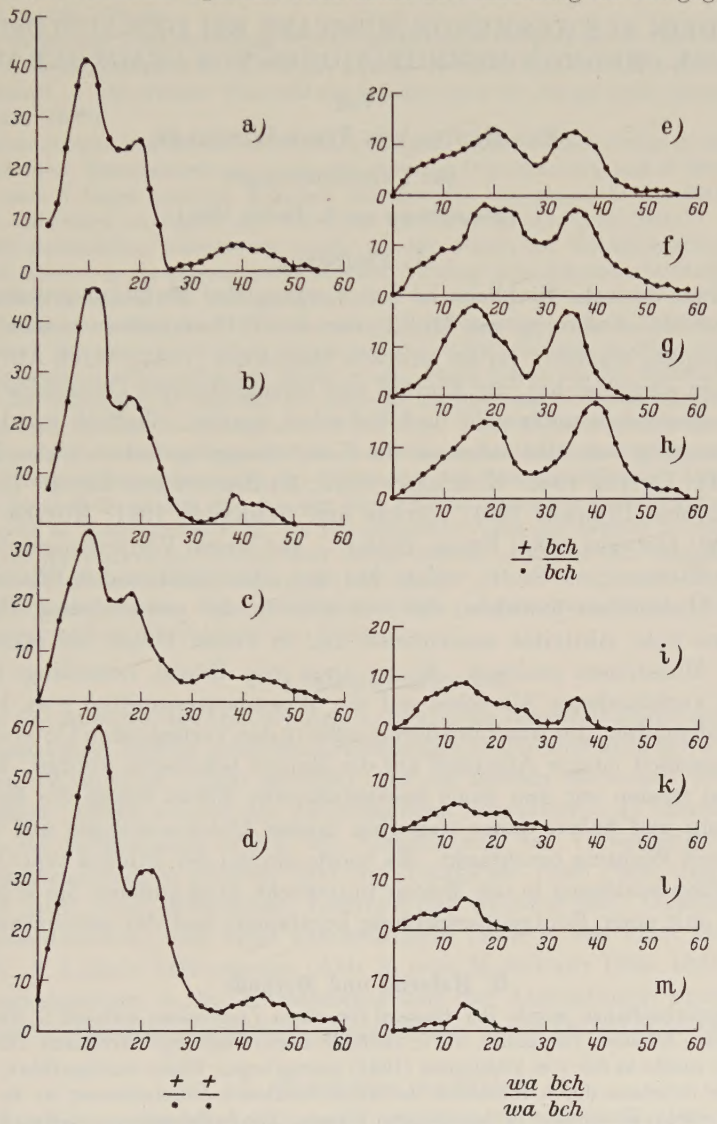

Abb. 3. Fluorometerkurven von je 4 Köpfen der Genotypengruppen

Literatur

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Prof. Dr. ALFRED KÜHN, Tübingen, Spemannstr. 34

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Aus dem Botanischen Institut der Universität Freiburg i. Br.

CODEIN ALS HEMMENDE SUBSTANZ BEI DER AUSLÖSUNG VON CHROMOSOMENMUTATIONEN VON *OENOTHERA**)

Von

EVA-BENITA VON TIELE-WINCKLER

Mit 7 Textabbildungen

(Eingegangen am 4. August 1955)

A. Einleitung

Für den Versuch, Einblicke in den Vorgang des Mutationsprozesses zu erlangen, hat die Auslösung von Mutationen durch Chemikalien wesentliche neue Gesichtspunkte ergeben. So ist, seitdem OEHLKERS (1943) durch Äthylurethan Mutationen ausgelöst hat, der Einfluß von verschiedensten Chemikalien auf das Mutationsgeschehen untersucht und diskutiert worden. Vielfach wurde so auch die Wirksamkeit von Alkaloiden auf die Kernteilungen getestet. (So z. B. ÖSTERGREN 1944; LETTRÉ 1946; KIHLMANN 1949; KIHLMANN und LEVAN 1949, 1951; OEHLKERS und LINNERT 1951; LETTRÉ und ALBRECHT 1951; NOVICK und SCILARD 1952; LINNERT 1952; ENGEL 1955.) — Bei diesen Verbindungen handelt es sich um pflanzeneigene Stoffe, welche bei den alkaloidführenden Pflanzen selbst keinerlei Mutationen bewirken, die sich aber in der menschlichen Physiologie durch eine hohe Aktivität auszeichnen und in vielen Fällen bei alkaloidfreien Pflanzen Mutationen auslösen. So ist auch von ENGEL neuerdings (1954) die Wirkung verschiedener Alkaloide auf die Wurzelspitzenmitose von *Vicia faba* untersucht worden. Im Anschluß daran sollte in den vorliegenden Untersuchungen die Wirksamkeit einiger Alkaloide auf die Meiosis bearbeitet werden. Bei diesen Versuchen fanden wir nun einen herausfallenden Effekt durch die Einwirkung von Codein und haben daher weiterhin unsere Untersuchungen auf diese neu konstatierte Wirkung beschränkt. Es wurde einmal der Einfluß von Codein auf die Mutationsauslösung in der Meiosis untersucht, zum anderen die Wirkung der Substanz mit einer Röntgenbestrahlung kombiniert und der gemeinsame Effekt studiert.

B. Material und Methode

Als Versuchspflanze wurde der Bastard *Oenothera (suaveolens sulfurea* × *Hookeri Freiburg) flavens* · *hHookeri* verwendet, wie in vielen früheren Freiburger Arbeiten. Die Versuchsanordnung wurde in der von OEHLKERS (1943) angegebenen Weise durchgeführt, indem die verwendete Substanz durch Einstellen der abgeschnittenen Infloreszenzen in die Lösungen appliziert wurde; Kontrollen in destilliertem Wasser. Die Infloreszenzen blieben 6 Tage lang bei konstanter Temperatur von + 10° und einer Luftfeuchtigkeit von etwa 70—75% in dem von OEHLKERS (1935) beschriebenen Kältethermostaten des Institutes stehen. Im Sommer 1953 wurden in die Klimakammer Leuchtröhren eingebaut (Philips Leuchtröhren TL). Durch diese wurde eine durchschnittliche Lichtstärke von 6000 Lux, gemessen in der Höhe der Infloreszenzgipfel, erreicht. Um eine Annäherung der Verhältnisse an die Freilandbedingungen zu erreichen, wurden die Pflanzen von 4—20 Uhr beleuchtet, während sie nachts im Dunkeln standen.

* Publikation nach einer Dissertation der Naturwiss.-mathem. Fakultät der Universität Freiburg i. Br.

Die Versuchslösung von Codeinum hydrochloricum cryst. (Merck-Präparat) wurde in der Konzentration von 1/500 molar hergestellt. Die Pflanzen der Röntgenversuche wurden 24 Std nach Einbringen in die Kältekammer geröntgt. Die Bestrahlungsbedingungen waren folgende: es wurden bei allen Versuchen jeweils 300 r (28,6 r/min) kontinuierlich verabreicht. Die HWS betrug 1,75 Al.

Am 6. Tage nach Versuchsbeginn wurden die Knospen morgens von 8—11 Uhr, eine Zeit, welche sich nach Zimmermann (1954) als günstig erwiesen hat, in Alkohol-Eisessig nach Carnoy fixiert. — Die weitere Verarbeitung erfolgte nach der Essigkarmin-Quetschmethode (Marquardt 1937).

Die Kombination von *flavens*- und *Hookeri*-Genom ergibt normalerweise in der Diakinese auf Grund ihrer Endenanordnung einen Ring von 4 Chromosomen und 5 Bivalenten. — Von den nach 6 Tagen fixierten Knospen befinden sich diejenigen, die die Diakinese-Konfiguration aufweisen, zu Beginn des Versuches im Pachytän (Linnert 1951).

Bei der statistischen Auswertung wurde in allen Fällen der Konfidenzschluß Neymans verwendet. Diesem heute allgemein anerkannten Verfahren liegt folgender Gedanke zugrunde: Wir interessieren uns in der vorliegenden Arbeit für den Erwartungswert der Aberrationsprozente und untersuchen die Differenz zweier Erwartungswerte, nämlich die von Kontrolle und Versuch. Nur wenn der Umfang des ausgewerteten Materials sehr groß ist, erhält man den Erwartungswert im arithmethischen Mittel genau. Steht jedoch nur ein Material begrenzten Umfangs zur Verfügung, so läßt sich ein Intervall angeben, das Konfidenzintervall, von dem behauptet wird, daß der Erwartungswert in ihm liege. Diese Methode enthält ein unvermeidbares Irrtumsrisiko. Im vorliegenen Falle wurde stets eine Normalverteilung angenommen und, der Konvention entsprechend, ein Irrtum mit der Wahrscheinlichkeit 0,05 zugelassen. In einem Spezialfalle, in dem eine Aussage über 2 Erwartungswerte zugleich gemacht werden sollte, wurde in Verallgemeinerung des Konfidenzintervalles ein Bereich in der Ebene angegeben, der die Punkte im Inneren einer Ellipse enthält. Außer in den Originalarbeiten ist das Problem auch anderweitig behandelt (Ihm 1954).

Mikroskopische Optik: Zeiss binokulares Stativ; Apochromat 90mal, Okulare 15 ×. Mikrophotographische Einrichtung von Zeiss.

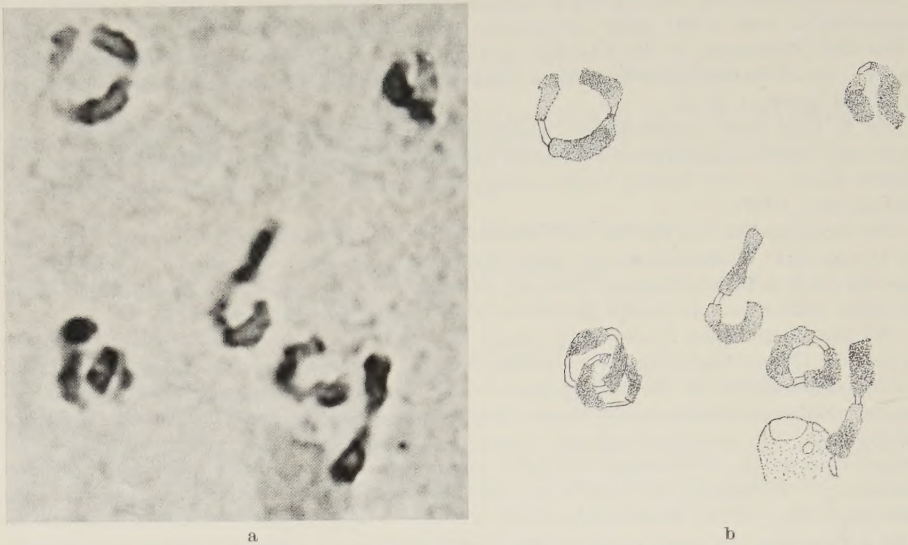
C. Experimenteller Teil

Über die Analysierbarkeit der Zellen, sowie die Beschreibungen der einzelnen Aberrationstypen wurden genauere Erörterungen in der ausführlichen Dissertation von Tiele-Winckler (1955) gemacht, auf die hiermit verwiesen wird. In der vorliegenden Arbeit wurden als Abweichungen konstatiert: 1. Translokationen, wie sie im wesentlichen durch die Veränderung der Verkettungsfiguren der Diakinesechromosomen erfaßbar sind (vgl. Oehlkers 1943, 1949; Oehlkers und Linnert 1949, 1951; Oehlkers, Linnert und Stange 1951; Oehlkers 1953). 2. Fragmentationen, und zwar chromosomale (Abb. 1) wie auch chromatidale (Abb. 2). 3. Lokale Achromasien (Abb. 3) (vgl. Marquart 1950, 1951). — Über die Schwierigkeiten in der Konstatierbarkeit der Aberrationen sowie über die Irrtumsmöglichkeiten ist ebenfalls Ausführliches in der Dissertation niedergelegt.

I. Einwirkung von Codein

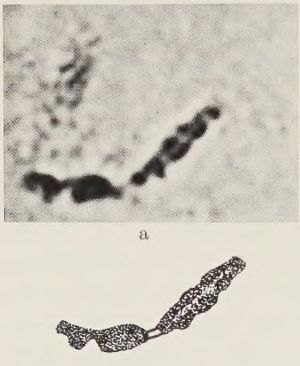
Codein gehört zu den Morphinalkaloiden, welche als Isochinolin- sowie als Phenantrenderivate aufgefaßt werden können. Es ist ebenso wie das Morphin ein tertiäres Amin, welches am Stickstoff eine Methylgruppe trägt. Ferner besitzt es eine alkoholische sekundäre Hydroxylgruppe und ein Phenolhydroxyl. Es unterscheidet sich von Morphin einzig dadurch, daß seine Phenolgruppe durch Methylierung blockiert ist.

1. Unspezifisch physiologische Störungen. Die Durchdringung der Infloreszenzen mit Codein war äußerlich dadurch sichtbar, daß sich nach 24 Std eine leichte Braunfärbung der Kelchspitzen einstellte. Die Knospen selbst waren jedoch ebenso turgeszent und gut fixierbar, wie die der Wasserkontrollen.



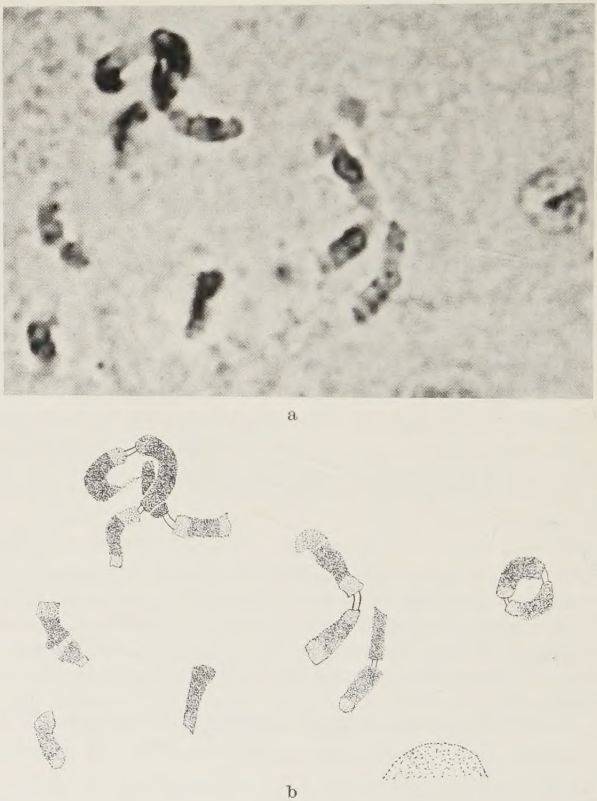
a b

Abb. 1a u. b. *Oenothera (suaveolens sulfurea × Hookeri Freiburg) flavens . ʰ Hookeri.* Vollständige Fragmentation nach Einwirkung von 300 r. a Mikrophotographie, b Schema. Vergr. 2600mal



a b

Abb. 2a u. b. *Oenothera (suaveolens sulfurea × Hookeri Freiburg) flavens . ʰ Hookeri.* Einzelchromosom mit chromatidaler Fragmentation nach Einwirkung von 300 r. a Mikrophotographie; b Schema. Vergr. 3000mal



a b

Abb. 3a u. b. *Oenothera (suaveolens sulfurea × Hookeri Freiburg) flavens . ʰ Hookeri.* Lokale Achromasie nach Einwirkung von Codein + 300 r. a Mikrophotographie; b Schema. Vergr. 2400mal

Die Beschaffenheit der Chromosomen war nach der Applikation von Codein nur wenig verändert. Es zeigte sich, daß dieselben im Verhältnis zur Wasserkontrolle geringfügig kompakter aussahen. Dieses schien uns durchgängig in allen Zellen konstatierbar. Der Nucleolus zeigte in Versuch und Kontrolle eine variable Ausbildung. Häufig wurde seine Auflösung in viele sehr kleine färbbare Körnchen beobachtet. Eine spezifische Veränderung im Aussehen des Nucleolus nach der Applikation von Codein konnte nicht festgestellt werden; jedoch wurde in diesen Versuchen das sog. Zerstäubungsstadium des Nucleolus auffallend bevorzugt angetroffen, während derselbe in der Wasserkontrolle in der Regel noch als kompakter Körper sichtbar blieb.

Die Werte für die Verklebungen in den analysierbaren Zellen, welche sich als Matrixbrücken zwischen 2 Chromosomen manifestieren, sind in Tabelle 1 aufgeführt. Sie stimmen in Versuch und Kontrolle überein; bei im Freiland fixierten Pflanzen werden Verklebungen nur äußerst selten gefunden. Auffallend ist die geringe Anzahl von Verklebungen bei den im Jahre 1952 durchgeführten Versuchen, zu welchen Seiteninfloreszenzen verwendet wurden.

2. Bindungsausfall und interstitielle Chiasmen. Über Bindungsausfall und interstitielle Chiasmen ist alles wesentliche in der Dissertation angegeben. Da sich nur die schon früher oft erwähnten Unterschiede fanden, nicht aber besondere Einwirkungen der verwendeten Substanz, ist eine genauere Erörterung hier nicht notwendig.

3. Häufigkeit der Chromosomen-Aberrationen. Über die Anzahl der gefundenen Aberrationen dieser Versuchsreihe gibt Tabelle 1 Auskunft. Fragmentationen wurden innerhalb dieser Versuchsreihe nicht gefunden. Auch an lokalen Achromasien konnte lediglich eine in dem 1953 durchgeführten Codeinversuch (II_2) konstatiert werden. Diese wurde aber in einer Zelle gefunden, die für die statistische Auswertung nicht herangezogen werden konnte. Somit stellen alle hier aufgeführten Aberrationen lediglich Translokationen dar. Die Zahl der Aberrationen ist in Prozent der analysierten Zellen angegeben.

Tabelle 1. *Häufigkeit der Aberrationen und Verklebungen nach Einwirkung von Codein im Vergleich mit Freilandfixierungen und H_2O-Kontrolle*

Jahr	Versuch	Einwirkung	Zahl der analysierten Zellen	Zahl der Aberrationen	Prozent der Aberrationen	Differenz der Aberrationsprozente (Kontrolle-Versuch)	Konfidenzintervall für den Erwartungswert der Differenz	Zahl der Verklebungen	Prozent der Verklebungen
1952	Freiland fixiert	—	442	7	1,6	—	—	2	0,005
1952	I_1	H_2O	208	10	4,81	—	—	5	2,4
1952	I_2	Codein	207	2	0,97	3,84	— 0,9 bis + 9,3	4	1,93
1953	II_1	H_2O	260	14	5,38	—	—	46	17,69
1953	II_2	Codein	260	4	1,54	3,84	1,7 bis 5,9	48	18,46

An den Anfang der Tabelle sind die im Freiland fixierten Pflanzen gestellt. Sie erbrachten eine spontane Mutationsrate von 1,6%, ein Wert, welcher mit den Befunden von Oehlkers und Linnert (1951) in guter Übereinstimmung steht (1,37%). Die Werte für die H_2O-Kontrolle lagen 1952 bei 4,81% und 1953 bei 5,83%. Diese Erhöhung der Aberrationsrate, welche die

Infloreszenzen, die den Versuchsbedingungen ausgesetzt sind, gegenüber den Freilandpflanzen aufweisen, ist wiederholt festgestellt worden (OEHLKERS, LINNERT 1951; STANGE 1951, MARQUARDT 1952) und wird auf die von den normalen Verhältnissen der Pflanzen abweichenden Bedingungen, die durch die Versuchsanordnung gegeben sind, zurückgeführt. Der Temperaturschock, der durch das Übertragen der Pflanzen in die Kältekammer zustande kommt und durch Nährsalzmangel bedingte Hungererscheinungen der Pflanzen könnten hierfür verantwortlich gemacht werden.

Im Vergleich mit der H_2O-Kontrolle wurde nach der Einwirkung von Codein eine Herabsetzung der Translokationsrate gefunden. Bei den im Jahre 1952 durchgeführten Versuchen ergab sich ein Aberrationsprozentsatz von 0,97%. Die Differenz der Aberrationsprozente der beiden Versuche ist 3,84. Für den Erwartungswert Δ der Differenz erhält man ein Konfidenzintervall, das in Tabelle 1 angegeben ist. Da $\Delta = 0$ hier im Konfidenzintervall liegt, ist keine Sicherung dieses Ergebnisses möglich. — Daher wurden die Versuche im folgenden Jahr wiederholt, wobei sich ein Prozentsatz von 1,54% Translokationen gegenüber dem der Kontrolle von 5,83% ergab. Aus dem Konfidenzintervall in Tabelle 1 ist ersichtlich, daß der Erwartungswert Δ der Differenz des Codeinversuches gegenüber der H_2O-Kontrolle deutlich von Null verschieden ist. Der Prozentsatz von 1,54% Translokationen stellt gegenüber der Kontrolle eine 3,8fache Erniedrigung der Mutationsrate dar und entspricht der im Freiland gefundenen spontanen Rate.

Tabelle 2. *Vergleich der Aberrationen nach Bestrahlung in Wasser und in Codein*

	Einwirkung	
	300 r in H_2O	300 r in Codein
Zahl der analysierten Zellen	300	260
Zahl der Aberrationen	44	25
Prozent der Aberrationen	14,67	9,62
Differenz Δ der Prozente (Kontrolle — Versuch)		5,1
Konfidenzintervall für Δ		—0,5 bis 10,5
Zahl der Translokationen	29	10
Prozent der Translokationen	9,67	3,85
Differenz Δ der Prozente (Kontrolle — Versuch)		5,8
Konfidenzintervall für Δ		1,9 bis 9,7
Zahl der vollständigen Fragmentationen	13	4
Prozent der vollständigen Fragmentationen	4,33	1,54
Differenz Δ der Prozente (Kontrolle — Versuch)		2,8
Konfidenzintervall für Δ		— 0,9 bis 6,3
Zahl der lokalen Achromasien	2	11
Prozent der lokalen Achromasien	0,67	4,23
Differenz Δ der Prozente (Kontrolle — Versuch)		3,6
Konfidenzintervall für Δ		2,1 bis 5,1
Zahl der Translokationen und Fragmentationen	42	14
Prozent der Translokationen und Fragmentationen	14	5,38
Differenz Δ der Prozente (Kontrolle — Versuch)		8,6
Konfidenzintervall für Δ		4,4 bis 12,8
Zahl der Fragmentationen und lokalen Achromasien	15	15
Prozent der Fragmentationen und lokalen Achromasien	5	5,77
Differenz Δ der Prozente (Kontrolle — Versuch)		—
Zahl der Verklebungen	39	52
Prozent der Verklebungen	13	20

Die Konfidenzintervalle sind der Übersicht halber als Strecken in Abb. 4 graphisch dargestellt. Projiziert man die Abszissenwerte Δ auf die einzelnen Strecken, so sieht man, daß

im Jahre 1952 Werte von Δ − 0,9 bis Δ + 9,3 zugelassen werden müssen. Das bedeutet, daß für den Erwartungswert der Differenz, der hier allein interessiert, negative und positive Zahlen einschließlich der Null zugelassen werden müssen. Eine negative Zahl bedeutet aber Mutationserhöhung, eine positive Zahl Mutationserniedrigung durch Codein, Null überhaupt keinen Effekt. Somit bleibt die Wirkung des Codeins nach dem Versuch von 1952 offen. Dagegen zeigt sich 1953 bei Werten von Δ zwischen + 1,7 und + 5,9, daß nur eine Erniedrigung in Frage kommt.

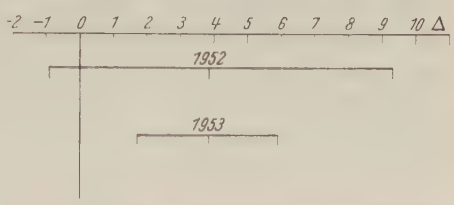


Abb. 4. Darstellung der Konfidenzbereiche für die Erwartungswerte Δ der Differenzen von Codeinversuchen und H_2O-Kontrollen in beiden Jahren. Abszisse: Werte von Δ

II. Kombination von Röntgenstrahlen und Codein

Nach diesen Versuchen schien es zweckmäßig zu untersuchen, ob die durch Codein in bezug auf die Mutationsauslösung bedingte, gewissermaßen stabilisierende Wirkung auf die Chromosomen sich auch dann ergeben würde, wenn die Zellen einem mutationsauslösenden Agens ausgesetzt würden. Eine Kombination von Codein mit mutagenen Chemikalien schien weniger zweckmäßig zu sein, da schwer zu sagen ist, welche Kombinationswirkungen der beiden Substanzen hierbei in der Zelle stattfinden. Sinnvoller schien daher eine Kombination von Röntgenstrahlen mit Codein zu sein, und zwar in Hinsicht darauf, ob durch seine Einflußnahme die bekannte Röntgenwirkung auf die Chromosomen reduziert wird.

1. Unspezifisch physiologische Störungen. Für die unspezifisch physiologischen Störungen ließ sich an Hand dieser Versuche zeigen, daß solche entsprechend der applizierten Lösung auftraten, und die Röntgenstrahlen auf diese Phänomene zum Teil keinen verändernden Einfluß hatten. Das betraf einmal das Aussehen der Chromosomen, die auch in diesen Versuchen nach der Einwirkung von Codein kompakter erschienen, und zum anderen das vermehrte Auftreten des Zerstäubungsstadiums des Nucleolus nach Codeineinwirkung. Mit dem Verklebungsphänomen verhielt es sich dagegen anders. Der Prozentsatz geht aus Tabelle 2 hervor. Hieraus ist ersichtlich, daß die Anzahl der Verklebungen im Codein + 300 r-Versuch gegenüber der in Wasser durchgeführten Röntgenkontrolle erhöht ist, und zwar steigt der Prozentsatz von 13% auf 20%. Ein solcher Effekt konnte nach der Einwirkung von Codein alleine nicht beobachtet werden.

2. Bindungsausfall und interstitielle Chiasmen. Es zeigte sich, daß der Bindungsausfall in dem mit Codein kombinierten Röntgenversuch etwas höher ist als in der geröntgten H_2O-Kontrolle, der Wert für interstitielle Chiasmen dagegen erniedrigt. Da diese Werte sich gegeneinander nicht sichern ließen, sind sie belanglos; ihre Einzeldaten können in der Dissertation eingesehen werden.

3. Häufigkeit der Chromosomen-Aberrationen. Während in den ungeröntgten Versuchen stets nur Zellen mit einer Aberration gefunden wurden, traten in den Röntgenversuchen ebenfalls Zellen mit mehr als einer Chromosomenmutation auf. Für einen quantitativen Vergleich wurde daher die Zahl der Aberrationen prozentual auf die Anzahl der analysierten Zellen berechnet und nicht die Zahl der Zellen mit Aberrationen angegeben.

Tabelle 2 bringt eine Übersicht über die gefundenen Aberrationsraten und ermöglicht einen Vergleich des Mutationsgeschehens in den beiden Versuchen.

Es ist daraus zunächst die durch Bestrahlung von 300 r in Wasser ausgelöste Aberrationsrate ersichtlich. Diese setzt sich aus Translokationen, Fragmentationen und lokalen Achromasien zusammen und beträgt für den Versuch H_2O + 300 r — 14,67%. Bei der Kombination von 300 r mit Codein zeigte es sich, daß die Aberrationsrate geringer war, und zwar beträgt sie nur 9,62%. Um die Möglichkeit der Sicherung der Ergebnisse anschaulich zu machen, wird in Abb. 5 dieselbe graphische Darstellung wie in Abb. 4 gebracht. In dieser sind wieder die Konfidenzintervalle für die einzelnen Werte als Strecken angegeben. Für den Erwartungswert Δ der Differenzen von den Aberrationen liegt in der Darstellung (vgl. auch Tabelle 2) wieder $\Delta - 0$ im Konfidenzbereich. Somit ist hier keine Aussage über die Sicherung dieses Ergebnisses möglich.

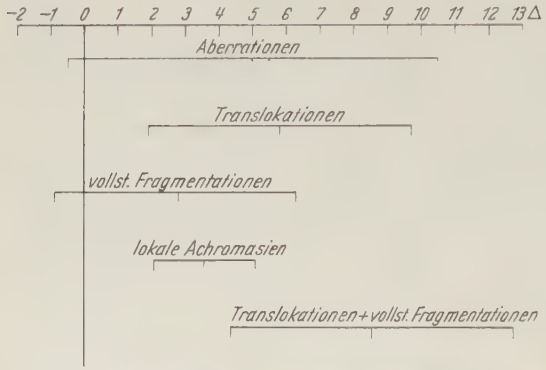

−2 −1 0 1 2 3 4 5 6 7 8 9 10 11 12 13Δ
Aberrationen
Translokationen
vollst. Fragmentationen
lokale Achromasien
Translokationen+vollst. Fragmentationen

Abb. 5. Darstellung der Konfidenzbereiche für die Erwartungswerte Δ der Differenzen von den Aberrationstypen nach Bestrahlung in Codein und H_2O. Abszisse: Werte von Δ

Für eine genauere Analyse wurden nun die Werte für die einzelnen Aberrationstypen getrennt berechnet. Hierbei ergab sich, wie aus der Tabelle ersichtlich ist, für den H_2O + 300 r-Versuch eine Translokationsrate von 9,67%, während hier der Wert für die Fragmentationen 4,33% beträgt. Lokale Achromasien wurden in diesem Versuch unter 300 ausgewerteten Zellen lediglich 2 gefunden, welches einen Prozentsatz von 0,67% ausmacht. Der Hauptanteil der Aberrationen wird demnach in diesem Versuch von Translokationen und vollständigen Fragmentationen gebildet.

Anders liegen die Verhältnisse bei dem Codein + 300 r-Versuch. Hier wurden nur 3,85% Translokationen gefunden, so daß wir es hier mit einer Erniedrigung der Translokationsrate gegenüber dem Kontrollversuch um das 2,5fache zu tun haben. Aus der graphischen Darstellung (Abb. 5) wie auch aus Tabelle 2 ist ersichtlich, daß kein Punkt im Konfidenzintervall liegt, der nicht deutlich von Null verschieden wäre, so daß die Behauptung, daß es sich um eine Erniedrigung der Translokationsrate handelt, voll begründet ist. Ferner ergab sich nach der Einwirkung von Codein eine Herabsetzung der Fragmentationen auf 1,54% und somit eine Erniedrigung gegenüber der Kontrolle um das 2,8fache, ein Wert, der freilich nicht statistisch gesichert werden konnte.

Gegenüber dem eben konstatierten Absinken gewisser Typen von chromosomalen Aberrationen ist es bedeutungsvoll, daß andere auch ansteigen. Den höchsten Wert erreichen die lokalen Achromasien. Der Prozentsatz beträgt 4,23%, so daß wir es hier mit einer Erhöhung gegenüber der in der Kontrolle gefundenen Rate auf das 6,3fache zu tun haben. Auch hier zeigt ein Blick auf das Konfidenzintervall in Abb. 5 (vgl. auch Tabelle 2), daß diese Behauptung begründet ist. Hieraus ist ersichtlich, daß die lokalen Achromasien in diesem Versuch nahezu die Hälfte aller gefundenen Aberrationen ausmachen.

Wird nun die Translokations- und Fragmentationsrate — ohne Berücksichtigung der Fragmentvorstufen — zusammengefaßt, so ergibt sich für die H_2O + 300 r-Kontrolle ein Prozentsatz von 14%, während die Rate nach der Einwirkung von Codein lediglich 5,38% beträgt, ein Wert, der der ungeröntgten H_2O-Kontrolle

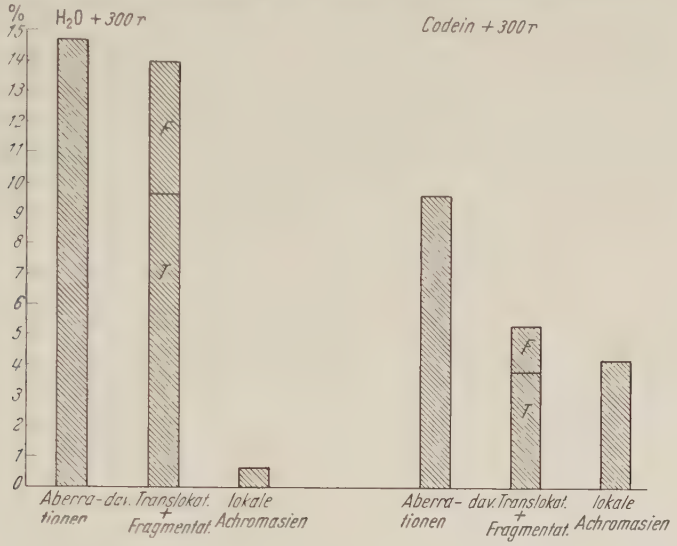


Abb. 6. Prozentsätze der Aberrationstypen nach Bestrahlung in Wasser und in Codein

entspricht und eine 2,6fache Erniedrigung der Kontrollrate darstellt; eine nach Betrachtung des Konfidenzintervalls ebenfalls gesicherte Aussage. — Weiterhin zeigt sich, daß bei Zusammenfassung von vollständigen und unvollständigen Fragmentationen die Zahlen für diesen Wert in Versuch und Kontrolle nahezu vollkommen übereinstimmen. — Die Ergebnisse dieser Versuche sind der besseren Übersicht halber noch einmal in Abb. 6 graphisch dargestellt.

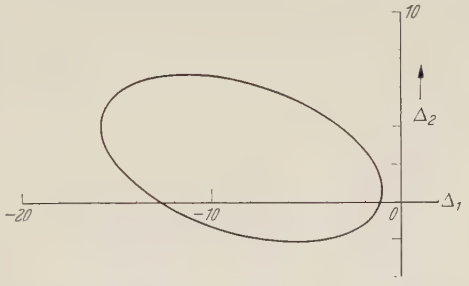


Abb. 7. Konfidenzbereich für die Differenzen der Erwartungswerte von Fragmentationen und Translokationen, Δ_1, und lokalen Achromasien, Δ_2, in Prozenten. (Codein + 300 r minus H_2O + 300 r)

Eine weitere Darstellung und Sicherung der Ergebnisse wird in Abb. 7 nach der Methode von Ihm (1954) gebracht. Da möglicherweise die Translokations- und Fragmentationsprozente mit den Prozenten der lokalen Achromasien korreliert sein können, ist es notwendig, einen Konfidenzbereich für den Erwartungswert des Differenzpaares anzugeben. Dieses wird gebildet aus der Zahl der Translokationen und Fragmentationen in Prozenten bei H_2O + 300 r minus der gleichen Zahl bei Codein + 300 r und derselben Differenz für die lokalen Achromasien. — Für die Anwendung dieser Methode war es allerdings notwendig, die Zahl der Pflanzen in dem Codein + 300 r-Versuch auf 10 und damit die der Zellen auf 200 zu reduzieren, um die Anzahl der Individuen in den beiden Versuchen gleich zu halten. In einem rechtwinkligen Koordinatensystem zeigt die Abszisse den Erwartungswert Δ_1 der Differenz von Translokationen und Fragmentationen, die Ordinate den Erwartungswert Δ_2 der Differenz der lokalen Achromasien in Prozenten an. Als Konfidenzbereich für dieses Differenzenpaar erhält man die dargestellte Ellipse. Aus der Darstellung ist ersichtlich, daß beide Werte

keineswegs zugleich Null sein können, und daß ebenfalls der Erwartungswert der Differenz der Translokationen und Fragmentationen, A_1, nicht Null ist. Ferner gibt die Lage der Ellipse an, daß bei starker Erniedrigung der Fragmentations- und Translokationsrate die Erhöhung des Prozentsatzes an lokalen Achromasien groß sein muß und umgekehrt: bei geringer Verminderung von Fragmentationen und Translokationen werden wenig lokale Achromasien gebildet. Fragmentations- und Translokationserniedrigung und Achromasienerhöhung sind also positiv korreliert.

Der qualitative Vergleich der Aberrationen in den beiden Versuchen ergibt also, daß die Erniedrigung der Aberrationsrate des Codein + 300 r-Versuches, welche im ganzen gesehen nicht statistisch gesichert werden konnte, ausschließlich auf einer Erniedrigung der Translokationen und echten Fragmentationen beruht. Das Verhältnis von Translokationen zu vollständigen Fragmentationen ist dabei in den beiden Versuchen nahezu gleich; es beträgt:

2,23 : 1 für H_2O + 300 r
2,5 : 1 für Codein + 300 r.

Diese Werte stimmen etwa mit den von Stange (1951) gefundenen Zahlen überein. Sie fand für die durch die gleiche Röntgendosis in Wasser ausgelöste Aberrationsrate ein Verhältnis von Translokationen zu Fragmentationen von 2,77 : 1. Der angegebene Prozentsatz für die Aberrationsrate im Codein + 300 r-Versuch erreicht nur deshalb einen Wert von 9,62%, weil die Zahl der Fragmentvorstufen in diesem Versuch gegenüber der Kontrolle stark erhöht ist.

Es kann somit eine gesicherte Erniedrigung der durch 300 r induzierten Translokations- und Fragmentationsrate durch die Einwirkung von Codein konstatiert werden, wohingegen die Bildung lokaler Achromasien durch diese Bedingungen gefördert wurde.

D. Diskussion

I. Die Einwirkung von Codein

In der vorliegenden Arbeit ist zunächst gezeigt worden, daß durch die Einwirkung von Codein die durch die Kältekammerbedingungen in reinem Wasser auftretende Aberrationsrate reduziert wurde. — Der erhöhte Mutationsprozentsatz der H_2O-Versuche im Vergleich mit den Freilandpflanzen kann nach Marquardt (1952) auf die erniedrigte Temperatur, der die Pflanzen ausgesetzt sind, zurückgeführt werden. Hierdurch soll eine Erhöhung der Sauerstoffkonzentration in der Zelle erzielt werden, welche für die aufgetretene Mutationsrate verantwortlich gemacht wird. Von Oehlkers und Linnert (1951) wird angenommen, daß im wesentlichen bei diesem Phänomen allgemeine Störungen der innerzellulären Bedingungen eine Rolle spielen. Man könnte somit annehmen, daß durch die Heraufsetzung des Sauerstoffgehaltes eine bestimmte Verschiebung von Stoffwechselprozessen innerhalb der Zelle gefördert wird, in deren Folge es zu Störungen kommt. Die zwischen der Behandlung des Pachytäns und der untersuchten Diakinese verstreichende Zeit würde genügend Spielraum für derartige Reaktionen lassen. — Hierbei könnte es möglich sein, daß solche Änderungen des Zellsystems auf der Beeinflussung von Fermenten, bzw. von fermentativ gesteuerten Prozessen beruhen. Von Woll (1953) ist gezeigt worden, daß eine Verschiebung im Gleichgewicht der Nucleinsäuren oder ihrer Bausteine Chromosomenmutationen, die als Fragmentationen getestet wurden, bewirken kann. — Die Schwierigkeit der Deutung der gefundenen Ergebnisse welche nach der Einwirkung von Codein erhalten wurden, liegt nun darin, daß über den Angriff der Substanz in der Zelle keine direkten Aussagen zu machen sind. So kann die Versuchslösung auf dem Wege durch die einzelnen Zellschichten bis zum Wirkungsort Veränderungen erfahren und in anderer Form und Konzentration zu den Pollenmutterzellen gelangen. Weiterhin läßt sich nicht entscheiden, ob diese in den Zellkern der reduzierenden Zellen hinein oder nur in das Zytoplasma gelangt.

Es kann deshalb hier zunächst nur angenommen werden, daß durch die Einwirkung von Codein die Verschiebung eines ursprünglichen Gleichgewichtszustan-

standes des zellulären Systems weitgehend auskompensiert wird. Rückschlüsse auf den möglichen Wirkungsmechanismus dieser Substanz können jedoch am ersten an Hand der Ergebnisse der mit Röntgenstrahlen durchgeführten Kombinationsversuche getroffen werden. Daher soll hierauf im folgenden Abschnitt zusammenhängend eingegangen werden.

II. Die Kombination von Röntgenstrahlen und Codein

1. Die Chromosomenmutationen. Bei der Kombination von Codein mit der Röntgendosis von 300 r zeigte es sich, daß die Translokations- und Fragmentationsrate gegenüber der Kontrolle herabgesetzt ist, die Zahl der lokalen Achromasien dagegen erhöht. Letztere sind von MARQUARDT (1950) als Fragmentvorstufen interpretiert worden, bei welchen eine lokal begrenzte Nucleinsäurestörung der Chromosomen vorliegt, und somit werden diese nicht als echte Brüche aufgefaßt. Sie können als schwächste Ausprägung einer Lösung des Chromonemaverbandes gelten. Die Röntgenwirkung findet demnach hier eine geringere Ausprägungsstärke, im Gegensatz zu den Erscheinungen der echten Fragmentationen und Translokationen. Die Rekombinationen können nach der STADLERschen Bruchhypothese (STADLER 1932) interpretiert werden. Danach wird angenommen, daß als primärer Vorgang das Brechen der Chromosomen erfolgt, woran anschließend sich die freien Bruchflächen zu einer neuen Konstellation vereinigen. Jede Translokation kann also als Ergebnis zweier Brüche aufgefaßt werden. – Da wir zu den echten Chromosomenaberrationen nur solche rechnen, die auf einen echten Bruch zurückgehen, können wir annehmen, daß durch die Einwirkung von Codein eine Reduktion der durch Röntgenstrahlen induzierten Chromosomenbrüche erreicht wurde.

Für diese den Röntgeneffekt reduzierende Wirkung des Codeins ist es nun von Bedeutung, daß sowohl die vollständigen Fragmentationen wie die Translokationen seltener auftreten und das Verhältnis dieser beiden Aberrationstypen bei der Herabsetzung das gleiche bleibt. Damit gibt es 2 Möglichkeiten, den Wirkungsmechanismus des Codeins zu erklären: 1. könnte durch den Einfluß von Codein eine Schutzwirkung auf die Chromosomen insofern ausgeübt werden, als die Zahl der durch den primären Absorptionsvorgang induzierten potentiellen Brüche verringert wird; 2. könnte nach erfolgtem Bruch die Rückrestitution der Chromosomen in den Ausgangszustand, die gewöhnlich die Zahl der sichtbaren Brüche stark herabsetzt, gefördert werden. Bei der Beantwortung dieser Fragen macht sich die Schwierigkeit geltend, daß sich diejenigen Brüche, die zum Normalzustand restituieren, nicht exakt bestimmen lassen.

Ob sich ein Bruchgeschehen als Fragmentation erhält oder zu einer Translokation oder einer Restitution zum Ausgangszustand weiterentwickelt, ist nach MARQUARDT (1950) von den mechanischen Spannungen im Chromosom und von der Anzahl und den gegenseitigen Lagebeziehungen der einzelnen potentiellen Brüche abhängig. Als potentieller Bruch wird hierbei wie gewöhnlich, diejenige Phase der Bruchentstehung bezeichnet, welche primär durch die Strahlen ausgelöst wird. Sind mehrere Bruchstellen miteinander in Berührung gekommen, ehe sich Restitutionen abspielen können, kommt es zur Translokation; hat ein potentieller Bruch hierzu keine Möglichkeiten gehabt, manifestiert er sich als Fragmentation. Man könnte nun hier annehmen, daß die Restitutionsbereitschaft der Brüche gesteigert wird, so daß weniger freie Bruchflächen erhalten bleiben und damit die Zahl der Fragmentationen und Translokationen herabgesetzt wird. Diese Annahme ist jedoch unwahrscheinlich, da keine Gründe für die Vermutung vorliegen, daß die Lagebeziehungen der Bruchstellen, welche wohl im wesentlichen für die Restitutionsbildung eine Rolle spielen, durch die Einwirkung von Codein verändert werden können.

Die erhöhte Anzahl lokaler Achromasien in diesem Versuch, welche man als Bruchvorstufen auffassen kann, spricht dafür, daß die durch die Absorptionsvorgänge bedingten strukturellen Veränderungen bei den unter Codeineinwirkung

stehenden Zellen nicht so eingreifender Natur sind. Wenn man die Anzahl der unvollständigen und vollständigen Fragmentationen zusammenfaßt, so ergeben diese in beiden Versuchen nahezu vollständig die gleichen Häufigkeiten. Daher liegt die Annahme nahe, daß die in dem mit Codein durchgeführten Versuch auftretenden lokalen Achromasien den vollständigen Fragmentationen des Kontrollversuches, nur in weniger ausgeprägter Form, entsprechen. Dafür spricht auch das Resultat, welches die statistische Behandlung mit Angabe eines Konfidenzbereiches ergeben hat. Denn ist die Erniedrigung der Fragmentations- und Translokationsrate durch die Einwirkung von Codein gegenüber der Kontrolle stark, so ist auch die Erhöhung des Prozentsatzes der lokalen Achromasien groß und umgekehrt. — So ließe sich denken, daß in dem Codeinversuch zum Teil keine voll fragmentierende Wirkung durch die ionisierenden Strahlenteilchen ausgelöst wurde; die Bildung vollständiger potentieller Brüche ist herabgesetzt, damit sind weniger freie Bruchstellen vorhanden und es erscheinen in der Diakinese weniger echte Fragmentationen und Translokationen. Wir möchten daher annehmen, daß die Ursache der herabgesetzten Aberrationshäufigkeit in diesem Versuch in dem primär induzierenden Ereignis und nicht in der anschließenden Folgereaktion der Restitutionsbildung gesehen werden könnte, obwohl sich eine endgültige Entscheidung an Hand der hier durchgeführten Versuche nicht treffen läßt.

Zu dem Versuch, den Mechanismus der Reduktion der Röntgenwirkung durch Codein aufzuklären, soll zunächst kurz auf die Probleme der biologischen Strahlenwirkung eingegangen werden.

2. Über die Wirkungsweise von Röntgenstrahlen. Bevor eine Erörterung der Schutzwirkung des Codeins und anderer Substanzen auf die Röntgenwirkung gegeben wird, sei kurz darauf hingewiesen, daß man zweierlei Mechanismen der Röntgenwirkung auf die lebende Zelle unterschieden kann: Einmal eine direkte Strahlenwirkung, wonach durch die Absorption der Strahlenquanten an ganz bestimmten Stellen, z. B. an den Chromosomen, eine Veränderung — etwa ein Bruch — herbeigeführt wird. Zum anderen kennt man eine indirekte Strahlenwirkung, bei welcher die erkennbaren Reaktionen über ein Bestrahlungszwischenprodukt, das in der Umgebung des reagierenden Systems entsteht, wirksam werden (vgl. Langendorff 1952). Als Beispiel einer indirekten Strahlenwirkung wird die Inaktivierung lebenswichtiger Stoffe und Fermente der Zelle und die bei teilungstätigem Gewebe anzutreffende Mitosehemmung (Heveš y 1949) angesehen. So haben sich in der Tat auch vielfache Veränderungen der Röntgenwirkung auf lebende Zellen durch den Einfluß verschiedener Agenzien, z. B. eine Herabsetzung der Sauerstoffspannung (Holthusen 1921), und eine Anzahl von Chemikalien nachweisen lassen (Langendorff 1950; Hollaender, Baker, Anderson 1951 a und b; Langendorff 1952; Langendorff, Koch und Sauer 1954, u. a.).

Die meisten Autoren, wie auch Langendorff (1952) sind freilich der Meinung, daß Vorgänge, wie der Bruch eines Chromosoms durch ionisierende Strahlen, wegen der hierzu erforderlichen hohen Energiekonzentration nur durch direkte Wirksamkeit herbeigeführt werden können. Auch von Sommermeyer (1952) wird die Ansicht vertreten, daß die Wirkung der Strahlen auf den Zellkern eine direkte ist, und zwar deshalb, weil die indirekte Strahlenwirkung als ein Ober-

flächeneffekt, die direkte aber als ein Volumeneffekt angesehen werden kann und letztere in ihrem Auftreten dem Volumen der Körper proportional ist. [Näheres siehe in der ausführlichen Dissertation von Tiele-Winckler (1955)].

Über wirksame Substanzen, welche die durch Bestrahlungen hervorgerufene Chromosomen-Aberrationsrate reduzieren, sind relativ wenig Ergebnisse bekannt. So ist von Mikaelsen (1952) für Glutathion an *Tradescantia palludosa* und von Forsberg und Nybom (1953) für Cystein an Versuchen mit *Allium cepa* nachgewiesen worden, daß diese Substanzen die strahleninduzierte Aberrationsrate herabsetzen.

Die in bezug auf die Auslösung von Chromosomenbrüchen schützende Wirkung des Cysteins wird so erklärt, daß diese Substanz wahrscheinlich auf die Nucleinsäuresynthese und ihren Umsatz wirkt. Es hat sich wiederholt zeigen lassen, daß unmittelbar nach einer Röntgenbestrahlung der Desoxyribosenucleinsäuregehalt des Zellkerns sich verändert, und zwar macht sich jede Strahlenwirkung auf die Zelle in einem Absinken ihres DNS-Gehaltes bemerkbar (Mitchell 1942; Ahlström, v. Euler und Hevešy 1944, 1945; Marshak 1948; Hevešy 1949; Holmes 1949, Darlington 1951). Man nimmt an (Langendorff 1950), daß hierbei vor allem Fermentsysteme, welche die Umwandlung von Ribonucleinsäure in Desoxyribosenucleinsäure steuern, angegriffen werden. Nach Gray (1954) wirken bereits kleine Bestrahlungsdosen auf die DNS-Synthese, und es wird geschlossen, daß von den Stoffwechselfunktionen der Zelle die DNS-Synthese bei weitem die radiosensibelste ist. Nach Langendorff (1950) kann nach einer Bestrahlung der DNS-Gehalt eines Gewebes auf $^1/_{10}$ des Normalwertes herabsinken, wobei der Gehalt an RNS gleichzeitig eine Erhöhung erfährt. Die RNS wird zunächst im Zytoplasma gebildet. Dem Umbau von Ribonucleotiden zu Desoxyribonucleotiden liegt ein reduktiver Vorgang zugrunde: es erfolgt ein Austausch einer OH-Gruppe mit einem H-Atom. Da aber eine Bestrahlung die Oxydationsvorgänge der Zelle begünstigt, ist verständlich, daß dieser Prozeß gehemmt wird. — Diese Tatsache erscheint nun besonders wichtig, wenn man bedenkt, daß die DNS ein Haupt- und wesentlicher Bestandteil der Chromosomen und für die Bildung von Chromosomeneiweiß von ausschlaggebender Bedeutung ist. Nach Darlington (1951) ist die Bruchempfindlichkeit der Chromosomen in denjenigen Stadien am größten, welche die geringste Nucleinsäurebeladung der Chromosomen besitzen. Für eine Reihe von Chromosomenaberrationen wird eine Nucleinsäureverarmung verantwortlich gemacht. Nach Marquardt (1950) scheint, daß die DNS-Anlagerung an die Chromosomen eine entscheidende Verfestigung ihres Längszusammenhaltes bewirkt. Und von Woll (1953) ist der Nachweis erbracht worden, daß eine Störung des Nucleinsäuregleichgewichtes Chromosomenmutationen hervorrufen kann.

Jedoch auch andere Enzyme, die am Oxydations-Reduktionssystem der Zelle beteiligt sind, werden wahrscheinlich von den Strahlen angegriffen. Nach Barron und Dickmann (1949a und b) werden vornehmlich in den Enzymen die SH-Gruppen durch Bestrahlung zu Disulfidbindungen oxydiert und werden damit im Unterschied zur Ausgangsform biologisch inaktiv. Es wird somit angenommen (Langendorff 1952), daß bei der Strahlenwirkung auf lebende Zellen die Inaktivierung der in großer Zahl in der Zelle vorhandenen SH-Enzyme eine wesentliche Rolle spielen. Da Cystein und Glutathion potentielle SH-Gruppen besitzen,

wird geschlossen, daß diese Substanzen die SH-Gruppen der Enzyme vor der Oxydation durch Bestrahlungsprodukte des Wassers schützen. Da diese Körper gleichzeitig stark reduzierende Wirkung besitzen, wird vermutet, daß sie in der Lage sind, ein durch die Strahlenwirkung inaktiviertes Enzym wieder in seine SH-Form zu überführen, wobei dessen SS-Brücken zu SH-Gruppen reduziert werden. Die Versuche von Forsberg und Nybom (1953) zeigten, daß das Cystein, wenn es in oxydierter Form, d. h. als Cystin, appliziert wurde, keine Schutzwirkung ausübte. Diese Tatsache unterstützt die Annahme, daß die Schutzwirkung dieser Körper nur bei der Gegenwart von freien SH-Gruppen in der Zelle stattfindet, daß also die SH-Gruppen dieser Substanzen den wirksamen Strahlenschutz bilden. — Auf der anderen Seite wurde hier jedoch gezeigt, daß das Vorhandensein einer potentiellen SH-Gruppe zur Strahlenschutzwirkung einer Substanz alleine nicht ausreicht, da verschiedene andere SH-haltige Verbindungen keinen solchen Effekt zeigen. Dieses wird jedoch so erklärt, daß die Verbindungen nicht im entsprechenden Maße in die Zellen eindringen.

In diesem Zusammenhang scheint es nun interessant, in Codein eine Substanz mit anscheinend ähnlichen Eigenschaften wie die der SH-haltigen Körper Cystein und Glutathion, aber von einem anderen Aufbau des Moleküls, gefunden zu haben.

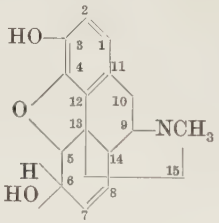
III. Der mögliche Wirkungsmechanismus des Codeins

Bei dem Versuch, den Wirkungsmechanismus dieser Substanz aufzuklären, ergeben sich hauptsächlich 2 Fragen:

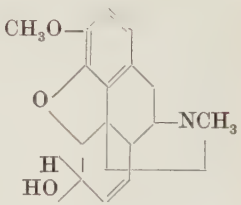
1. Die nach der Bedeutung der Konstitution des Moleküls,
2. die nach dessen Angriffspunkt in der geschädigten Zelle.

1. Die Bedeutung der Konstitution des Codeinmoleküls. Auf die nahe Verwandtschaft dieses Alkaloids mit Morphin ist bereits im experimentellen Teil hingewiesen worden: Morphin und Codein unterscheiden sich lediglich durch eine phenolische Hydroxylgruppe, die bei Morphin frei und bei Codein durch Methylierung blockiert ist:

Morphin



Codein



(nach Robinson)

Von Oehlkers und Linnert (1951) ist nun an dem gleichen *Oenotheren*-Bastard, der in den vorliegenden Versuchen verwendet wurde, gezeigt worden, daß durch Morphin eine bedeutsame Erhöhung der Mutationsrate erzielt wird. Die Wirkungsweise dieser beiden Substanzen scheint demnach entgegengerichteter Art zu sein. Es müßte danach in diesem Zusammenhang geschlossen werden, daß die mutationsfördernde Wirkung von Morphin auf die Wirksamkeit dieser phenolischen OH-Gruppe zurückzuführen ist, und die antimutagene Wirkung von

Codein auf den Ersatz des phenolischen Wasserstoffes durch die CH_3-Gruppe. Rein chemisch weisen Codein und Morphin weitgehend die gleichen Reaktionen auf. Beide Substanzen wirken reduzierend und neigen zu Polymerisation (Brühl 1900). Codein unterscheidet sich von Morphin dadurch, daß es leichter löslich, besonders auch in Wasser, ist. Auch in ihrer physiologischen Aktivität unterscheiden sich die beiden Substanzen nachdrücklich voneinander. — Es könnte angenommen werden, daß Codein — im Gegensatz zu Morphin — eher die Fähigkeit besitzt, in seiner eigentlichen Form in die Zelle einzudringen.

Damit ergibt sich die Frage nach dem Angriffspunkt des Codeins in der Zelle. Hierzu sollen zunächst die vorliegenden Ergebnisse mit den in der Literatur vorhandenen interessierenden Arbeiten verglichen werden.

2. Zur Frage nach dem Angriffspunkt des Codeins in der Zelle. *a) Vergleich mit Untersuchungen über Codeineinwirkung in der Mitose.* Engel (1954) untersuchte den Einfluß verschiedener Alkaloide, darunter auch Codein, auf die Wurzelspitzenmitose von *Vicia faba*. Durch Codein wie durch Morphin wurde hier eine Förderung der Fragmentations- und Achromasierate bewirkt, wobei durch die Einwirkung von Codein die Achromasierate stärker heraufgesetzt wurde als durch Morphin. Außerdem wurde nach der Einwirkung von Codein zunächst eine Mitosehemmung und darauf eine vorübergehende Förderung festgestellt. Auch von Dustin (1939) ist eine Störung des Mitoseablaufs durch Codein festgestellt worden, während nach Untersuchungen von Lettré (1946) am Mäuse-Ascites-Tumor keine Mitosegiftwirkung von Codein sowie von Morphin, konstatiert werden konnte.

Nach diesen Ergebnissen könnte nun zunächst angenommen werden, daß das Codein möglicherweise eine Veränderung in der Ablaufgeschwindigkeit der meiotischen Stadien bei *Oenothera* bewirkt. Das ist jedoch unwahrscheinlich, da eine vollständige Übereinstimmung der Diakinesehäufigkeiten in Versuch und Kontrolle gefunden wurde. Daraus ist zu schließen, daß die Veränderung der Mutationsrate nach Codeineinwirkung wahrscheinlich nicht im Zusammenhang mit einer Stadienverschiebung steht.

Die in bezug auf die Mutationsauslösung gegensätzlichen Ergebnisse der Arbeit von Engel mit den vorliegenden Befunden könnten somit nur auf ein unterschiedliches Reaktionsvermögen der beiden Objekte und der beiden Kernteilungen, Mitose und Meiosis, zurückgeführt werden. Es wäre begreiflich, daß sich bei verschiedenartigem Substrat unterschiedliche Reaktionen ergeben. Auch im Tier-Versuch wurde beobachtet, daß einzelne Objekte unterschiedlich auf strahlenschützende Substanzen reagieren: Agenzien, die bei Ratten eine schützende Wirkung hatten, zeigten keine solche bei Mäusen (Langendorff).

b) Vergleich der Codeinwirkung mit der Schutzwirkung von Cystein. Eine auffallende Übereinstimmung unserer Ergebnisse finden wir dagegen mit denen von Forsberg und Nybom (1953). Sie fanden nach der Einwirkung von Cystein eine Herabsetzung der durch 150 r ausgelösten Fragmentationsrate, wobei die Häufigkeit der Fragmentationen von in Wasser und Cystein bestrahlten Wurzeln ein durchschnittliches Verhältnis von 1:2,4 ergab. Wir fanden nach der Einwirkung von Codein eine Herabsetzung der Fragmentations- und Translokationsrate um das 2,6fache. Diese Werte sind demnach in exakt der gleichen Größenordnung und stimmen ebenfalls in dieser Hinsicht mit der von Thoday und Read (1947) gefundenen Herabsetzung der Aberrationsrate nach einer Röntgenbestrahlung von 143 r in Stickstoff und Sauerstoff überein.

Zum anderen wurde bei der Kombination von Cystein und Röntgenstrahlen eine verstärkte Verklebungstendenz der Chromosomen festgestellt. Auch in diesem Punkt stimmen unsere Ergebnisse mit denen von Forsberg und Nybom überein: Die Häufigkeit der Verklebungen war nach Kombination von Codein und Röntgenstrahlen gegenüber der Wasserkontrolle von 13% auf 20% erhöht. Nach der Einwirkung von Codein allein konnte jedoch keine Erhöhung der Verklebungen festgestellt werden. Dieses scheint demnach kein spezifischer Effekt des Codeins zu sein.

c) Die unspezifisch physiologischen Störungen. In dem Zusammenhang zwischen Aberrationsrate und unspezifisch-physiologischen Störungen läßt sich nun jedoch möglicherweise ein Hinweis auf die Wirkungsweise der untersuchten Substanz finden. In den mit Codein durchgeführten Versuchen war ein starkes Vorherrschen der unspezifisch-physiologischen Störungen auffällig. — Die erhöhte Anzahl von Verklebungen in dem mit Codein durchgeführten Röntgenversuch könnte dafür sprechen, daß die Herabsetzung der Aberrationsrate möglicherweise mit Veränderungen im Nucleinsäuregehalt der Chromosomen parallel geht. — Bei dem Verkleben der Chromosomen handelt es sich um eine Veränderung der Oberflächenbeschaffenheit derselben. Dieses Phänomen konnte unter verschiedenen ungewöhnlichen Bedingungen auf eine Überladung mit Nucleinsäure zurückgeführt werden (Darlington 1951). Nach Koller (1951) zeigen häufige Verklebungen an, daß eine übermäßige und sehr schnelle Produktion von Nucleinsäuren erfolgt ist. Demnach scheint ein unmittelbarer Eingriff in den Nucleinsäurestoffwechsel die Ursache der Verklebungen zu sein. Für eine relativ stärkere Nucleinsäurebeladung der Chromosomen würde auch das leicht kompaktere Aussehen derselben nach Einwirkung der verwendeten Substanz sprechen, ein Phänomen, welches in allen mit Codein durchgeführten Versuchen beobachtet wurde. Denn nach Darlington (1951) sind die Chromosomen infolge einer Überbeladung mit Nucleotiden überspiralisiert und überfärbt. — In diesem Punkt finden wir nun eine annähernde Übereinstimmung zwischen unseren Ergebnissen und denen von Engel (1954). Dieser fand eine antimutagene Wirkung der Alkaloide Yohimbin und Pilokarpin. Verbunden mit einer Herabsetzung der Fragmentations- und Achromasierate war eine Erhöhung der Verklumpungen. Diese beruhen neben einer Zusammenballung der Chromosomen auf einer Verklebung ihrer Oberflächen.

Für die Annahme, daß Beziehungen solcher Art vorliegen, spricht auch die Tatsache, daß der Nucleolus in den mit Codein durchgeführten Versuchen auffallend häufig im Zerstäubungsstadium gefunden wurde, während er in der Kontrolle meist noch als kompakter Körper sichtbar war. Daß der Nucleolus zu Vorgängen des Nucleinsäurestoffwechsels in Beziehung steht, wobei er wahrscheinlich als eine Art Depot von Ribonucleinsäuren dient, ist bekannt (Kühn 1955). Normalerweise werden auch einige der Aminosäuren seiner Histone zum Aufbau der Desoxyribonucleinsäuren herangezogen. Unser Befund könnte somit dafür sprechen, daß möglicherweise in den Codeinversuchen ein beschleunigter oder verstärkter Abbau der Nucleolarsubstanz erfolgte, welches für eine Beziehung der verwendeten Substanz zum Nucleinsäurestoffwechsel spricht.

d) Die Reaktionsweise von Codein. Nach dem hier dargestellten möchten wir somit schließen, daß das Codein möglicherweise über den Nucleinsäurestoffwechsel der Zelle insofern eine Wirkung ausübt, als eine verstärkte DNS-Beladung der Chromosomen erfolgt. An das Chromosomeneiweiß würden durch dessen

Wirksamkeit im verstärkten Maße Nucleinsäuren angelagert und somit eine Verfestigung derselben bewirkt, wodurch die Bruchfähigkeit herabgesetzt wird. Nach Woll (1953) sind Stellen, die sich durch eine geringe Nucleinsäurebeladung auszeichnen, Orte bevorzugter Fragmentationsbereitschaft. Auch ist wiederholt die Ansicht vertreten worden, daß mutagene Agenzien erst auf dem Umweg über den Nucleinsäurestoffwechsel die Chromosomen-Aberrationen auslösen (Darlington und Koller 1947; Oehlkers 1949; Marquardt 1950; Darlington 1951; Koller 1951; Woll 1953). Von Marquardt (1950) ist darauf hingewiesen worden, daß sich zwischen Pachytän und Diplotän ein diffuses Stadium einschieben kann, in welchem sich eingreifende Veränderungen im Nucleinsäurestoffwechsel vollziehen (siehe auch Tischler 1943). Diese Phase zeichnet sich durch eine starke DNS-Verarmung aus. Hiernach ist verständlich, daß durch die Einwirkung mutagener Agenzien auf dieses Stadium auch noch Chromosomenaberrationen ausgelöst werden können. — Durch die Wirksamkeit des Codeins würde somit einmal eine Verschiebung im Gleichgewicht der Nucleinsäuren verhindert, welche durch reines Wasser hervorgerufen wird bzw. auf die durch die tiefe Temperatur zustande gekommene Erhöhung des Sauerstoffgehaltes zurückzuführen ist. Zum andern wird durch die Substanz die dieses System stark schädigende Strahlenwirkung reduziert.

Somit würde aber die Wirkungsweise einer solchen Schutzsubstanz dafür sprechen, daß der Schädigung des Nucleinsäurestoffwechsels durch die Strahlen für die Häufigkeit des Auftretens strahleninduzierter Aberrationen eine Bedeutung beigemessen werden muß. Da aber eine Schädigung des Nucleinsäuresystems als Folge einer indirekten Strahlenwirkung angesehen werden kann, wäre somit gleichzeitig die Bedeutung des indirekten Effektes einer Bestrahlung auf die Auslösung von Chromosomenbrüchen ersichtlich. So könnte angenommen werden, daß zumindest ein Teil der strahleninduzierten Chromosomenveränderungen durch indirekten Effekt bedingt werden. Dem strahlenempfindlichen Volumen würden danach nicht nur die Chromosomen, sondern die ganzen Zellen angehören. — Die Ergebnisse zeigen, daß die Häufigkeit des durch die Strahlen erzeugten potentiellen Bruches nicht nur von der direkten Wirkung der eingestrahlten Energie abhängig ist, sondern auch von den physiologischen Bedingungen der Zelle.

Zusammenfassung

Es wurde der Einfluß von Codein auf die Meiosis von *Oenothera (suaveolens sulfurea* × *Hookeri Freiburg) flavens* · ^{h}*Hookeri* untersucht. Dazu wurde einmal die Wirkung von Codein allein auf die Mutationsauslösung getestet, zum anderen die Substanz mit einer Röntgendosis von 300 r kombiniert.

1. Codein setzt die in aqua dest. durch die Versuchsbedingungen entstehende Aberrationsrate auf das Maß der im Freiland auftretenden spontanen Rate herab.

2. Die durch 300 r induzierte Translokations- und Fragmentationsrate wird durch Codein reduziert.

3. Die Bildung lokaler Achromasien wird bei Kombination von Codein und 300 r im Vergleich zur Kontrolle erhöht.

4. Die Chiasmenbildung wird durch keine der Versuchsbedingungen verändert.

5. Die antimutagene Wirkung von Codein wird zu Vorgängen des Nucleinsäurestoffwechsels in Beziehung gebracht.

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AUTORADIOGRAPHIC STUDIES OF *DROSOPHILA* GONADS FOLLOWING THE FEEDING OF ^{14}C LABELLED FORMALDEHYDE

By

William D. Kaplan and Stephen R. Pelc

With 5 figures in the text

(Eingegangen am 12. August 1955)

Introduction

The pattern of sensitivity which formaldehyde elicits from the germ cells of *Drosophila* is the most striking aspect of its mutagenic properties. The work of Auerbach[1, 2, 3] indicates that a restricted zone of sensitivity moves posteriorly along the larval testis as larval development progresses. When males are fed formaldehyde food during the first day of larval life the mutation rate is high among the offspring derived from the treated sperm first to be utilised, decreases in the second brood and falls to the control rate in the brood. Following treatment on the last day of larval life mutation rate is low in the first brood, rises to a maximum in the second or third brood and then falls to control level. Transitional patterns are obtained as a result of treatment during the second and third days of larval life.

No mutations are produced as a result of feeding formaldehyde food to larval females. Moreover, formaldehyde food fails to induce mutations in the germ cells of either male or female adults, whereas the injection of formaldehyde solution into the abdomens of males does result in mutations.

Auerbach and Moser[4] conclude that mutations are induced by formaldehyde preferentially in the long prophase growth stage preceding the first meiotic division, the auxocyte stage. The basis for this selectivity is not known but might be attributed to a differential penetration of the formaldehyde into germ cells at various stages or might be due to a differential reactivity with the chemical.

It was believed that the use of ^{14}C formaldehyde might clarify this point and indicate the basis for the sensitivity that has been shown to exist. The results seem to favor the existence of a differential response to the presence of formaldehyde — but this idea must be accepted with certain reservations.

Method

The feeding method used by Auerbach[3] was adopted. The medium, 13% corn meal, 15% molasses, 10% killed brewer's yeast in a 1.5% solution of agar in tap water, referred to as "fortified medium" in Auerbachs paper, was prepared in the usual way and then ^{14}C-labelled formaldehyde was added after the food had been allowed to cool but before it had solidified. To 40 ml. of food 1.3 ml. ^{14}C formaldehyde containing 28.8 mg. $H^{14}CHO$ per ml., activity 14 uc/mg. were added. Therefore, a total of 37.4 mg. of $H^{14}CHO$ with a total activity of 523.6 uc. were present in the food. Thereafter, 4 ml. of the mixture were poured into individual vials and the food was allowed to solidify. The age of larvae is computed from the time of egg-laying.

Fifteen larvae were placed in each vial. Two vials received larvae that were 24 hours old, the second two vials received 48 hour old larvae, the third two vials larvae that had reached the 72 hour stage. Fifteen 96 hour larvae were placed in one vial.

Starting at six hours after the larvae were placed in the radioactive food and at six hour intervals thereafter, three larvae were removed from each vial. They were fixed in acetic acid and alcohol, 3:1, punctured with a fine tungsten needle and allowed to remain in the fixative for two hours, after which they were placed in 70% alcohol. There was, thus, obtained a series of larvae of four stages of development, each stage of which had been in contact with labelled formaldehyde food for 6, 12, 18 or 24 hours.

Larvae not removed from vials were permitted to complete development and, upon eclosion, the gonads were removed from the adults and fixed in acetic acid and alcohol.

In order to determine whether formaldehyde enters into the gonads after adults have fed upon formaldehyde food two groups of adults, ten males and ten females were placed in separate vials, kept in contact with the labelled food for three days and then the gonads were removed and fixed.

Dehydration followed fixation and after the tissues had reached absolute alcohol a double embedding system in celloidon and paraffin was carried out according to the Peterfis technique. The tissues were sectioned at 5 microns thickness and the autoradiography was carried out by the stripping-film technique as described by Doniach and Pelc[5] and later discussed by Pelc and Howard[6].

Observations

A. Males

I. 24 hour larvae: After six hours on radioactive food a weak autoradiograph is observable in the gonads. The intensity of activity increases only slightly during the next six hour interval. After 18 hours there is moderate activity and at the end of 24 hours there is a very strong autoradiograph. In all cases the activity is present throughout the entire testis. There appears to be no selective absorption. The activity observed in the testes is the same as that in the other tissues of the larvae.

The slight autoradiograph obtained during the first 12 hours is an indication that only a small amount of formaldehyde reaches the germ cells during this period, perhaps because the newly hatched larvae do not begin to eat immediately. Genetic results obtained by Auerbach and Moser[4] indicate that the mutation rates produced during the second 12 hour portion of the first day were higher than during the first 12 hours period and approached that obtained for the full 24 hours' treatment. This may reflect an absence of sensitive cells during the first 12 hours. However, the autoradiographs indicate that this difference in mutation rate may depend, not upon a differential response to the presence of formaldehyde, but rather to the delay on the part of the chemicals in reaching the sensitive germ cells.

II. 48 hour larvae: There is a general autoradiograph throughout the testis as was found in the 24 hour larvae. Here, again, the intensity of the activity within the testis parallels that in the other tissues of the organism. Also, intensity increases with time in contact with the radioactive food although the spectrum of intensity is not as wide as in the case of the 24 hour larvae. After 6 hours there is a moderate autoradiograph which increases by 18 hours to almost the maximum intensity observed after 24 hours. There are no genetic data available to determine whether the mutation frequency following the first 12 hours is less

than the frequency following feeding during the second 12 hour period of the second day of larval life.

III. 72 hour larvae: The testes of all four classes of 72 hour larvae present the same picture. There is a strong autoradiograph as a result of feeding upon the ^{14}C formaldehyde food, the intensity of which does not appear to increase with time. The autoradiograph produced after six hours on radioactive food is equal in intensity with that produced after 12, 18 or 24 hours. This would seem to indicate that the 72 hour larva eats voraciously during this period and that a maximum concentration of labelled compounds enters the larval tissue. When a certain concentration has been reached, no more enters the individual cells. In this connection, it is interesting to note that the intensity of activity is higher above the gut regions where food is present than within the tissues of the organism.

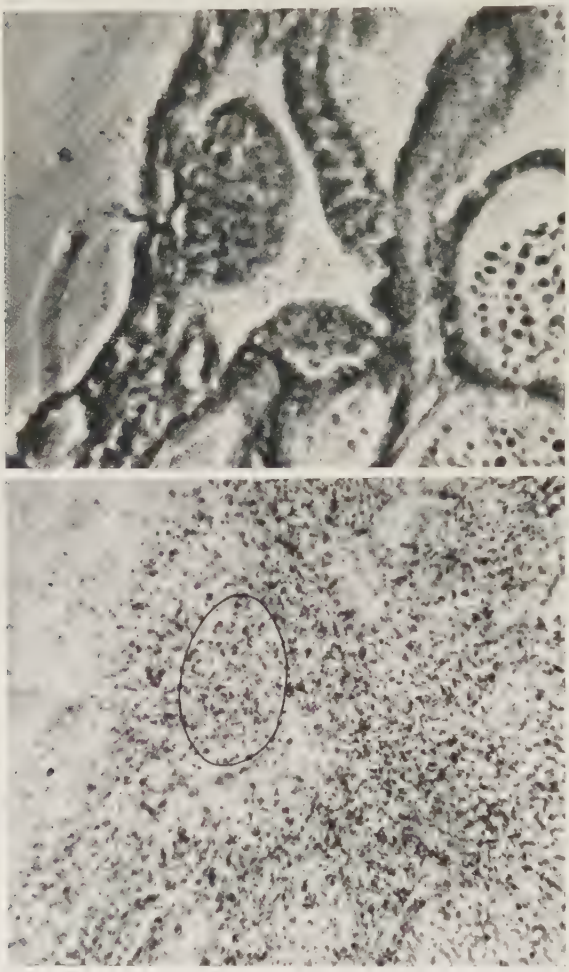

Fig. 1A and B. Testis. 24 hour larva removed after 12 hours contact with labelled food. A. Phase contrast. B. Bright field picture of autoradiograph

The activity above the anterior end of the gonad — the region of spermatogonial cells — appears to have a heavier autoradiograph than the more posterior cells. This difference may be more apparent than real inasmuch as the gonial cells are markedly smaller at this stage than the older germ cells, a size difference which does not exist in younger larvae (compare Figs. 2 and 3). If the concentration of compounds bearing the ^{14}C atom is the same in each cell, the number of reduced photographic particles occupying a smaller volume above the gonial cells would appear more densely aggregated. Moreover, this observation, if it should represent a greater penetration of formaldehyde in this region of the gonad than in any other region, strengthens the idea that differential penetration is not at the basis of the observed pattern of sensitivity, for genetic results are contrary to this notion inasmuch as mutation rates in the fourth brood obtained from

males treated as larvae at 72 hours have returned to control levels. Under the conditions of AUERBACHS experiments cells which were spermatogonia at the

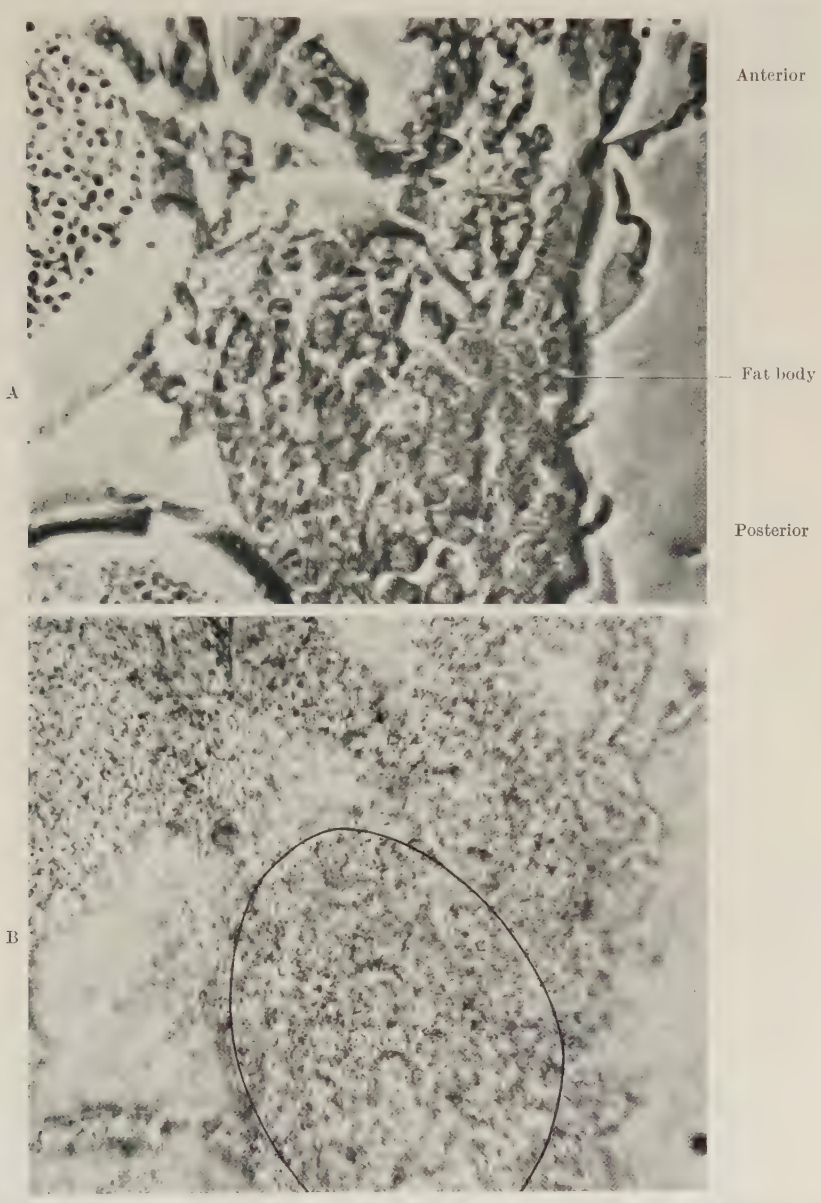

Fig. 2 A and B. Testis. 48 hour larva removed after 18 hours contact with radioactive food. A. Phase contrast. B. Autoradiograph. × 320

time of treatment would provide the fertilising sperm for not earlier than the fourth brood.

It has been observed that the oldest germ cells of the 72 hour larva are no longer the sensitive ones[4], although in younger larvae these same auxocytes are the most sensitive cells. Since, clearly, strong activity was observed above the

nuclei of these auxocytes, one must rule out the possibility of non-penetration of the mutagenic agent into the nuclei. Apparently, then, as was pointed out by AUERBACH and MOSER[4] on the basis of genetic results, the sensitive period does no extend over the whole of the auxocyte stage but is limited to the early growth period of this cell type.

It might also be pointed out at this time that the low mutation rates observed following treatment of males at 72 hours, compared to those rates observed for 24 and 48 hour larvae, is not the result of smaller amounts of formaldehyde penetrating the gonads, relative to the amounts absorbed by gonads of younger larvae.

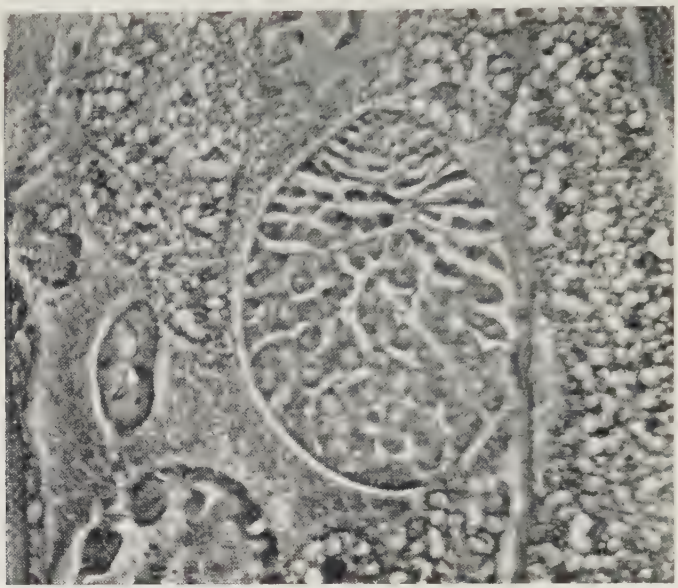

Fig. 3. Testis. 72 hour larva removed after 12 hours on labelled food. × 110

at least insofar as the autoradiograph technique serves to indicate. The intensity of autoradiographs for the 72 hour larvae present on food for 24 hours was as great as in the case of younger larvae.

IV. 96 hour larvae: There was a great deal of variation in the physiological age of larvae placed on labelled food at this stage. It was observed that some larvae immediately crawled up the glass side of the vial, others remained on the surface of the food, and some few burrowed in the food for a short time before pupation. Accordingly, only three larvae present on the food for 18 hours, were fixed and studied for the 96 hour series. The autoradiographs obtained from these 3 larvae were all weak but were consistent in the uniformity of pattern. Again there appeared to be no selective absorption. In the case of 96 hour larvae the low mutation rates observed may well be due to the small intake, although this cannot be the whole story. The rate of mutation observed at this stage is usually higher than for 72 hour larvae.

V. The gonads of adults developing from larvae which were placed on radioactive food: In all four series some larvae were permitted to complete development

after having fed upon the ^{14}C formaldehyde food. Following eclosion the adult gonads were removed, fixed and the autoradiograph technique followed through.

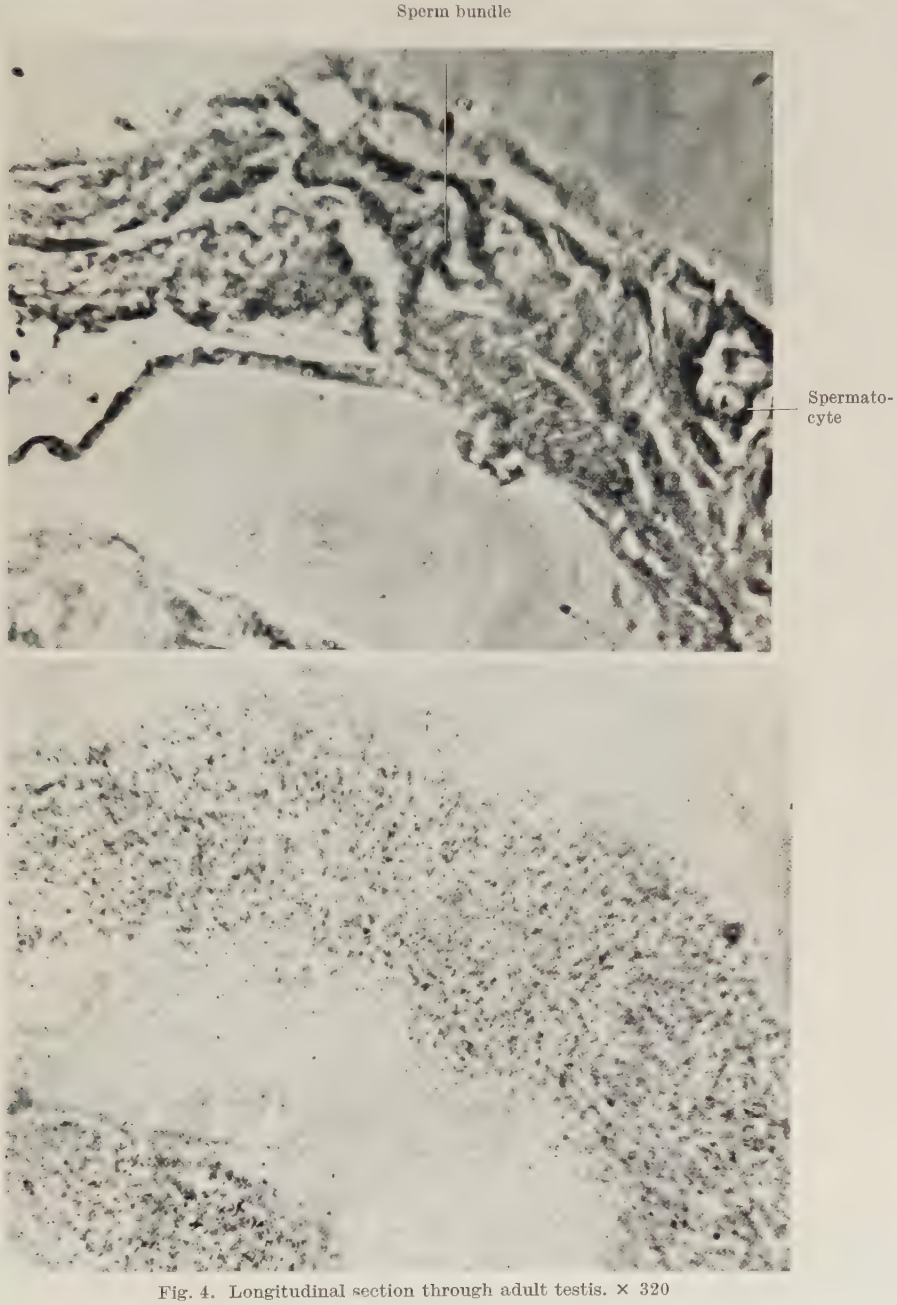

Fig. 4. Longitudinal section through adult testis. × 320

In every case, except for larvae placed on food at 96 hours, the adult testis showed a clear strong autoradiograph with equal activity over germ cells in all stages of development. In the case of the 96 hour larvae some testes showed no

activity, while in two instances there was a weak autoradiograph. One would expect, from these observations, that there would be a great deal of variability

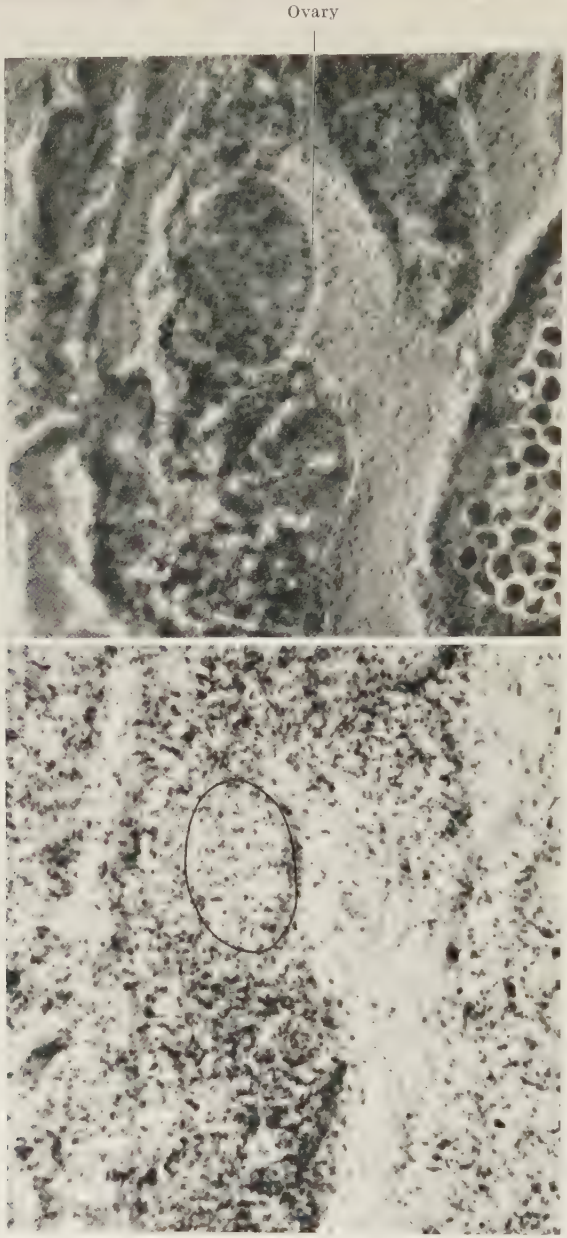

Fig. 5. Ovary. 24 hour larvae in contact with labelled food for 18 hours. A. Phase contrast. B. Autoradiograph. × 480

in mutation rates between individual males treated at this larval stage — a variability which reflects differences in the amount of food consumed, which in turn reflects variability in stage of development reached due to pretreatment differences that become more pronounced as larval development advances. This point has been discussed elsewhere[4].

In a second series of experiments larvae were placed on ^{14}C formaldehyde food for 24 hours, then transferred to fresh non-labelled food to complete development. Following eclosion, the adult gonads were fixed and autoradiographed. The picture presented was similar to the previously described series. Radioactivity was present in all cells from spermatogonia to mature sperm and the intensity of activity was equivalent to that observed for comparable developmental stages.

This observation indicates that the amount of formaldehyde present in the gonads at the time of pupation may have been much the same whether larvae had been in contact with the food for 24 hours or whether they had been placed upon the food at 24, 48 or 72 hours of age and then left there to complete development. In work which is, as yet, unpublished, Auerbach has found that 24 hours of treatment produces the same genetic effect as longer treatment. This work serves to indicate that Auerbachs observation may mean that given a certain concentration of formaldehyde in the food there is a maximum level of concentration to which formaldehyde may reach within the cells of the larval gonad.

B. The female gonad

Female larvae were subjected to the same experimental treatments as have been described for male larvae in the above sections. In all cases, regardless of the age at which the larvae were placed upon the radioactive food, or the length of time they remained in contact with it, positive autoradiographs were obtained in the region of the developing gonad — as Fig. 5 demonstrates. This observation indicates that the lack of response to formaldehyde on the part of the female gonad may not be due to the inability of the chemical to penetrate into the maturing germ cells, but rather suggests that the response is different from that of the male germ cells. However, it must always be understood that the observed autoradiograph indicates only the presence of labelled carbon and gives only limited information regarding the chemical compound of which it is a part.

C. Adults in contact with labelled formaldehyde food

Male and female adults were placed in vials containing labelled formaldehyde food and after three days the gonads were removed, fixed and prepared for autoradiography.

In the case of both sexes, marked activity was observed over germ cells of all stages of development. As in the case of larval females, and with the same reservations, it may be said that the failure of formaldehyde to induce mutations when fed to Drosophila adults is not due to lack of penetration but to lack of the response which characterises its action upon larval male germ cells.

Discussion

Formaldehyde is a highly chemically reactive substance. It is known to enter into many types of chemical reactions with the resultant formation of a diversity of compounds. Therefore, a wide variety of chemical reactions may take place between formaldehyde and various elements within the food substrate, within the digestive system of the larvae, within the haemolymph, within the cytoplasm of the germ cells and within the nuclei of the germ cells. Consequently, when an autoradiograph is observed one may conclude only that the ^{14}C atom is present. The compound in which it is present remains unknown.

It may well be that the ^{14}C has entered the germ cell nucleus as part of the formaldehyde molecule, or it may have entered as a C atom in some other compound.

These facts must be considered in interpreting the results of the work herein described. What has been observed would seem to indicate that a differential penetration does not lie behind the pattern of sensitivity to the mutagenic action of formaldehyde as measured by genetic techniques. Assuming that the ^{14}C is part of a molecule other than formaldehyde by the time it reaches the gonads, there is no reason to assume it reaches the various regions of the gonad in different forms. The most likely assumption, therefore, is that germ cells of different stages of development respond differently to the mutagenic agent derived from the technique of formaldehyde feeding or that they are able to inactivate the formaldehyde or the derived mutagen.

It is difficult to explain why the female gonad remains refractory to the action of this mutagen especially as a positive autoradiograph is obtained after larvae have fed on food containing radioactive formaldehyde. This may be due to compounds in the ovary which are missing in the testis, compounds which inactivate formaldehyde at least so far as its mutagenic action is concerned. Or it may reflect the absence of auxocyte stages. No definite hypothesis can, however, be forwarded at this time.

Summary

Male and female larvae of *Drosophila melanogaster* at 24, 48, 72 and 96 hours of age were placed upon a standard type of food to which ^{14}C labelled formaldehyde had been added. They were left to feed for specified periods of time and then removed and autoradiographs were prepared. The technique used demonstrated that formaldehyde enters the male and female gonad with penetration equally in all regions. The differential sensitivity demonstrated by genetic techniques seems, thus, to be a consequence of differential response to the presence of formaldehyde rather than to differential penetration.

It has been pointed out that a positive autoradiograph indicates the presence of ^{14} C atoms but does not tell anything about the compounds in which they are present.

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Aus dem Botanischen Institut der Universität Köln

GENETISCHE UND ENTWICKLUNGSPHYSIOLOGISCHE UNTERSUCHUNGEN ÜBER DIE SELBSTSTERILITÄT VON *RAPHANUS RAPHANISTRUM*

Von

M. Kroh

Mit 4 Textabbildungen

(Eingegangen am 19. August 1955)

A. Einleitung

Die selbststerilen Cruciferen folgen hinsichtlich der genetischen Grundlage der Selbst- und Kreuzungssterilität keinem einheitlichen Schema. Die Kreuzungsergebnisse von *Capsella grandiflora* und *Raphanus sativus* veranlaßten Riley (1936) und Tatebe (1944) zu der Annahme zweier hemmender Genpaare bzw. Allelenserien, zwischen denen Epistasie auftreten kann; außerdem scheint sporophytische Determination des Pollens vorzuliegen. *Capsella grandiflora* und *Raphanus sativus* lassen sich daher dem homostyl-sporophytischen System zuordnen, dem auf Grund der genetischen Untersuchungen von Gerstel (1950) an *Parthenium argentatum*, Hughes und Babcock (1950) an *Crepis foetida* und Crowe (1954) an *Cosmos bipinnatus* folgende Charakteristika zugesprochen werden konnten: Die Selbst- bzw. Kreuzungssterilität ist durch ein *S*-Gen mit zahlreichen Allelen bedingt. Der Pollen steht unter sporophytischer Kontrolle. Zwischen den Allelen des Pollens einerseits und denen des Griffels andererseits kann Dominanz auftreten, sie können aber auch unabhängig voneinander wirken. Die Kreuzungsresultate an *Brassica oleracea* und *Cardamine pratensis* ließen Kakizaki (1930) und Beatus (1934) eine andere genetische Grundlage der Selbststerilität annehmen. Es scheint bei diesen Cruciferen eine hemmende und eine fördernde Allelenserie vorzuliegen, zwischen denen bestimmte Epistasieverhältnisse bestehen. Der Pollen ist gametophytisch determiniert. *Brassica oleracea* und *Cardamine pratensis* können daher weder zu dem oben kurz charakterisierten homostyl-sporophytischen noch zu dem homostyl-gametophytischen System (*Nicotiana*-Schema) gezählt werden. Das homostyl-gametophytische System basiert ja ausschließlich auf der Wirkung *eines* Sterilitätsgens mit zahlreichen Allelen und gametophytischer Determination des Pollens.

Da die Selbststerilität der bisher untersuchten Cruciferenarten auf verschiedenen genetischen Grundlagen beruht, wurde in den vorliegenden genetischen Untersuchungen geprüft, welcher Vererbungstyp der Selbststerilität, Kreuzungssterilität und Pseudofertilität bei *Raphanus raphanistrum* vorliegt. Dies war notwendig, da in physiologischen Experimenten die Wirkung der *S*-Gene bei der Pollenkeimungs- bzw. Pollenschlauchhemmung untersucht werden sollte.

Die selbststerilen Cruciferen sind durch ein gemeinsames physiologisches Merkmal ausgezeichnet: Die Inkompatibilitätsreaktion findet bereits auf der Narbe statt. Ein großer Teil der Pollenkörner keimt nach Selbstbestäubung nicht aus. Die wenigen Schläuche, die gebildet werden, bleiben in der oberflächlichen

Papillenschicht der Narbe stecken. Nach Berichten von Sears (1937) und Tatebe (1939) ist die Sterilitätsreaktion bei *Brassica oleracea* und *Raphanus sativus* ausschließlich auf die *oberste Narbenregion beschränkt*; das tiefer liegende Narbengewebe und der Griffel vermögen keine Hemmung auf Pollenkeimung und Schlauchwachstum auszuüben. Besonders die letztgenannte Tatsache ließ die Cruciferen als entwicklungsphysiologisches Versuchsobjekt in den Vordergrund rücken. Sollte sich auch für *Raphanus raphanistrum* herausstellen, daß die Pollenschlauchhemmung nach Selbstbestäubung auf die *Narbenoberfläche beschränkt* ist, so mußte er sich gut dazu eignen, die Hemmreaktion *in vitro* ablaufen zu lassen. Der Nachweis einer spezifischen Pollenschlauchhemmung *in vitro* war von Bedeutung, da er die Grundlage für eine Erfassung der chemischen Natur der Sterilitätsreaktion darstellt.

B. Material und Methoden

Der zur Aussaat verwandte Samen entstammte Früchten weißblühender *Raphanus raphanistrum*-Pflanzen (2n = 18), die seit 1949 im Botanischen Garten Köln durch künstliche Fremdbestäubung vermehrt worden waren. Herkunft der Pflanzen: Frohnschwand im südlichen Schwarzwald.

Die Herstellung von Klonen erfolgte durch Pfropfung von Seitentrieben auf junge Rosenkohlpflanzen, da sich die vegetative Vermehrung durch Stecklinge als ungünstig erwiesen hatte. Sobald die Jahreszeit es erlaubte, wurden die jungen Pflanzen mit den Töpfen auf einem mit Glas überdachten Stück Freiland in die Erde eingesenkt. Die Blühdauer der *R. raphanistrum*-Pflanzen betrug maximal 3 Monate. Eine Übersicht über Herkunft und Bezeichnung der zu den Untersuchungen verwendeten Pflanzen bringt Tabelle 1.

Tabelle 1. *Herkunft und Bezeichnung der Versuchspflanzen*

Aussaat-Termin	Herkunft der Samen	Bezeichnung der Pflanzen
Januar 1952	N 232 × fremd	E 1,2...
Juli 1952	E 5 × E 4	F II 1,2...
Juli 1952	E 4 × E 5	F I 1,2...
Juli 1953	F II 3 × selbst.	G 3/ 1,2...
Dezember 1953	F II 3 × selbst.	G_1 3/ 1,2...
Dezember 1953	F II 13 × F II 3	G I 1,2...
Januar 1954	F II 4 × selbst.	G 4 1,2...

Kurz vor dem Einsetzen der Blüte wurden die Pflanzen zum Schutz gegen Insektenbestäubung in Glasbatistsäckchen eingebeutelt. Eine Kastration der Blüten erwies sich als überflüssig, da die Pflanzen vor jeder Kreuzbestäubung auf ihre Selbststerilität hin geprüft wurden und eine Beeinflussung der Fremdbestäubung durch eigenen Pollen nicht zu vermuten war. Die Entfernung der Staubblätter wurde nur bei den Blüten vorgenommen, die zu dem Versuch dienten, die Pollenkeimungsrate in vivo nach Selbst- und Fremdbestäubung zu bestimmen. In diesem Fall erfolgte die Kastration etwa 24 Std vor der Knospenöffnung.

Zur Feststellung der Pollenkeimungsrate *in vivo* wurden die selbst- bzw. fremdbestäubten Narben nach bestimmten Zeitabständen in Karminessigsäure (mit Eisen) übertragen. Nach 12 Std wurden sie 15 min lang in dem gleichen Gemisch gekocht, anschließend auf einen Objektträger in einen frischen Tropfen Karminessigsäure überführt und die Narben unter einem Deckglas gequetscht.

Verschiedene Bestäubungsmethoden und die methodische Durchführung der Pollenkeimungsversuche *in vitro* werden im experimentellen Teil dargelegt.

Die statistische Auswertung der Versuchsergebnisse erfolgte mit Hilfe der χ^2-Methode und des t-Testes nach Student. Die entsprechenden P-Werte wurden in den Tafeln von Fisher und Yates (1938) aufgesucht.

C. Untersuchungsergebnisse

I. Genetische Untersuchungen

1. Die Genetik der Selbst- und Kreuzungssterilität. Um einen Einblick in die genetische Grundlage der Selbst- und Kreuzungssterilität von *R. raphanistrum*

zu bekommen, wurden 12 Pflanzen aus der Nachkommenschaft der Kreuzung FII 13 × FII 3 selbstbestäubt und reziprok in allen möglichen Kombinationen gekreuzt. Die beiden Elternpflanzen FII 13 und FII 3 waren streng selbststeril. Da die Zahl der Blüten, die eine Pflanze hervorbringt, bei den einzelnen Individuen erheblich variierte, war bei vielen Kombinationen die Anzahl der bestäubten Blüten gering. Sie schwankt zwischen 2 und 8. In Tabelle 2 sind die Resultate der Bestäubungen aufgeführt.

Tabelle 2. *Resultate der Selbst- und Kreuzbestäubungen in der Nachkommenschaft der Kreuzung FII 13 × FII 3*

Gruppe	♂		A								B		C	
	Untergruppe		I				II		III	IV	I	II	I	II
♀		Pflanzen Nr. G I	1	9	11	23	8	20	14	12	3	7	10	15
A	I	1	—	—	—	—	—	—	—	—	+	+	+	+
		9	—	—	—	—	—	—	—	—	+	+	+	+
		11	—	—	—	—	—	—	—	—	+	+	+	+
		23	—	—	—	—	—	—	—	—	+	+	+	+
	II	8	—	—	—	—	—	—	—	—	+	+	+	—
		20	—	—	—	—	—	—	—	—	+	+	+	—
	III	14	—	—	—	—	—	—	—	—	+	+	—	+
	IV	12	—	—	—	—	—	—	—	—	+	+	—	—
B	I	3	+	+	+	+	+	+	+	+	—	—	—	—
	II	7	+	+	+	+	+	+	+	+	—	—	—	+?
C	I	10	+	+	+	+	+	+	+	+	+	—	—	—
	II	15	—	—	—	+	—	+	+	+	+	+	—	—

Die Plus-Zeichen in dem Schema bedeuten, daß die Kreuzung positiv, die Minus-Zeichen, daß sie negativ ausgefallen ist. In 18 von 144 Kombinationen konnte das Ergebnis einer Kreuzung nicht eindeutig als + oder — bezeichnet werden, da nicht alle Bestäubungen der betreffenden Kreuzung das gleiche Ergebnis aufwiesen. Es wurde festgesetzt, daß in solchen Fällen eine Kreuzung positiv gewertet wird, wenn mindestens die Hälfte der durchgeführten Bestäubungen positiv ausfällt.

a) Einteilung der Nachkommenschaft aus der Kreuzung FII 13 × FII 3 in Gruppen und Untergruppen. Die Selbstbestäubungen sämtlicher 12 Pflanzen (Diagonale!) fielen negativ aus. In Tabelle 2 wurde versucht, die Pflanzen auf Grund ihrer gleichen bzw. verschiedenen Kreuzungsresultate in Gruppen einzuteilen.

Zunächst konnten 3 Sterilitätsgruppen aufgestellt werden, die mit A, B und C bezeichnet werden sollen. Innerhalb jeder Gruppe sind alle Pflanzen kreuzungssteril. Die Pflanzen der Gruppe A lassen sich mit denen der Gruppe B reziprok fertil kreuzen. Betrachten wir die Kreuzungsresultate von Gruppe A × C, C × A; B × C und C × B, dann müssen wir feststellen, daß sich die Pflanzen einer Gruppe *nicht mehr einheitlich* gegenüber einer anderen Gruppe verhalten. Innerhalb einer jeden Gruppe müssen sich die Pflanzen daher noch einmal in Untergruppen gliedern, und zwar muß sich auf Grund der Kreuzungsresultate die Gruppe A in 4, die Gruppen B und C in je 2 Untergruppen aufteilen. Die Untergruppen sollen mit römischen Ziffern benannt werden.

b) Die genetische Deutung der Kreuzungsresultate. Die Kreuzungsresultate in Tabelle 2 lassen sich nicht durch das *Nicotiana*-Schema von East (1925) erklären, da die Pflanzen innerhalb der Gruppen A, B und C in bezug auf ihre Sterilitätsallele kein einheitliches Material darstellen. Es wurde daher versucht, ein genetisches Schema aufzustellen, das den Anforderungen der Kreuzungsergebnisse in Tabelle 2 gerecht wird. Dieses Schema basiert auf folgenden Voraussetzungen:

1. Vorhandensein von 2 Sterilitätsallelenserien, die mit S und Z bezeichnet werden sollen. Jede Serie enthält 4 Allele S_1, S_2, S_3, S_4, und Z_1, Z_2, Z_3, Z_4.

2. Das Verhalten des Pollens ist sporophytisch bestimmt.

3. Innerhalb der beiden Allelenserien liegen zwischen den einzelnen Allelen im Pollen folgende Dominanzverhältnisse vor: $S_1 > S_2$, $S_2 > S_4$, $S_3 > S_4$; $Z_1 > Z_2$, $Z_2 > Z_4$.

(Im folgenden wird unter einem S_xS_y-Pollenkorn ein Korn verstanden, das von einer Pflanze der genetischen Konstitution S_xS_y gebildet worden ist. Es enthält selbstverständlich nur ein Allel, S_x oder S_y. Auf Grund seiner sporophytischen Determination ist es aber von beiden Allelen der Mutterpflanze geprägt, von denen jedoch in erster Linie nur ein Allel, nämlich das dominante, wirksam ist.)

4. Zwischen der S- und der Z-Serie bestehen bestimmte Beziehungen, die an Hand von 3 Beispielen erläutert werden sollen:

a) Trifft ein S_1S_2-Pollenkorn auf einen S_3S_4-Griffel, dann tritt unabhängig von dem Vorhandensein der Z-Allele immer ein Fruchtansatz auf.

b) Trifft ein S_1S_2-Pollenkorn auf einen S_1S_3-Griffel, dann tritt immer eine Hemmung des Schlauchwachstums ein, ganz gleich welche Z-Allele sich gegenüberstehen.

c) Trifft ein S_1S_2-Pollenkorn auf einen S_2S_3-Griffel, dann müßte an sich ein Fruchtansatz stattfinden, da der Pollen durch das S_1-Allel determiniert wird, welches im Griffel fehlt. In diesem oder einem hierzu analogen Fall können jedoch die beiden Z-Allele Z_1 und Z_2, falls sie vorhanden sind, in die Reaktion eingreifen und das Verhalten des Pollens insofern bestimmen, als Z_1 epistatisch über S_1 und S_2, und Z_2 epistatisch über S_2 ist. In diesem Beispiel kann also die Reaktion zwischen Pollen und Griffel auch durch die Z-Allele bestimmt werden. Die Allele Z_3 und Z_4 haben keine epistatische Wirkung über die S-Allele.

In ihrer Wirkungsweise verhalten sich die Z-Allele analog den S-Allelen.

Zur besseren Übersicht sind in Tabelle 3 die Kreuzungsresultate noch einmal zusammengefaßt. In dieser Tabelle sind die Gruppen durch S- und Z-Allele gekennzeichnet.

Wenn wir den beiden Elternpflanzen F II 13 und F II 3 die genetische Konstitution $S_2S_3Z_1Z_4$ bzw. $S_1S_4Z_2Z_3$ geben, dann können nach den oben aufgeführten Voraussetzungen den Untergruppen die in Tabelle 3 aufgezeichneten genetischen Konstitutionen zugeordnet werden. Aus Tabelle 3 ersieht man, daß die Untergruppen einer Gruppe durch das Vorhandensein gleicher S-Allele charakterisiert sind. Die 4 Untergruppen von Gruppe A enthalten die Allele S_3S_4, die beiden Untergruppen von Gruppe B die Allele S_1S_2 und die beiden Untergruppen der Gruppe C die Allele S_2S_4. (Eine 4. Gruppe, die auf Grund der Kreuzung die

Tabelle 3. *Zusammenfassung der Bestäubungsresultate aus Tabelle 2, verbunden mit einer Bestimmung der genetischen Konstitution der einzelnen Gruppen bzw. Untergruppen*

Gruppe	Konstitution ♀	♂ Untergruppe	A S_3S_4 Z_3Z_4 I	A S_3S_4 Z_2Z_4 II	A S_3S_4 Z_1Z_3 III	A S_3S_4 Z_1Z_2 IV	B S_1S_2 Z_3Z_4 I	B S_1S_2 Z_1Z_2 II	C S_2S_4 Z_1Z_2 I	C S_2S_4 Z_2Z_4 II
A	S_3S_4 Z_3Z_4	I					+	+	+	·
	S_3S_4 Z_2Z_4	II					·	+	+	
	S_3S_4 Z_1Z_3	III					+	+	·	·
	S_3S_4 Z_1Z_2	IV	·				·	·	·	·
B	S_1S_2 Z_3Z_4	I	·	·	·	·			·	·
	S_1S_2 Z_1Z_2	II	·	·	·	·	·		·	· ?
C	S_2S_4 Z_1Z_2	I	·	·	·	·	·	·		
	S_2S_4 Z_2Z_4	II	·	·	·	·	·	·	·	

Allele S_1S_3 enthalten mußte, konnte nicht aufgefunden werden.) Die Untergruppen einer Gruppe unterscheiden sich in ihren Z-Allelen.

Im folgenden sollen die Resultate von zwei Kreuzungen an Hand der genetischen Konstitution ihrer Kreuzungspartner interpretiert werden.

Das Gen, das durch sein dominantes bzw. epistatisches Verhalten gegenüber den drei übrigen Allelen ausgezeichnet ist und die Reaktion des Pollens auf einer Narbe bestimmt, ist unterstrichen; ferner wird nur die Allelenserie aufgeführt, die bei der Reaktion zwischen Pollen und Griffel eine Rolle spielt.

Als Beispiele mögen die Kreuzungen AII × BII und AII × CII dienen. Die Untergruppe AII ($S_3S_4Z_2Z_4$) läßt sich mit Untergruppe BII ($S_1S_2Z_1Z_2$) fertil kreuzen, da sich die beiden Untergruppen in beiden S-Allelen unterscheiden. Die Kreuzung AII ($S_3S_4Z_2Z_4$) × CII ($S_2S_4Z_2Z_4$) müßte auf Grund der beteiligten S-Allele ebenfalls positiv ausfallen. CII-Pollen, der S_2S_4 enthält, müßte auf AII-Narben, die S_3S_4 enthalten, auskeimen und zu den Samenanlagen durchwachsen. Hier tritt aber der oben beschriebene Fall ein, daß der CII-Pollen nicht durch das S_2-Allel determiniert ist, sondern durch das Z_2-Allel, welches stärker als S_2 wirkt. Da die $S_3S_4Z_2Z_4$-Pflanzen der Untergruppe AII gleichfalls das Z_2-Allel enthalten, muß die Bestäubung dieser Gruppe mit Z_2-determiniertem Pollen der Untergruppe CII negativ ausfallen.

Entsprechend dieser beiden Beispiele lassen sich mit Hilfe der oben angeführten Voraussetzungen die Kreuzungsresultate in Tabelle 3 bis auf eine einzige Ausnahme erklären. Die Ausnahme stellt die Kreuzung BII × CII dar, die entgegen unserer Erwartung positiv ausfiel. Dieses Kreuzungsresultat stützt sich jedoch nur auf 2 Bestäubungen, von denen eine einen Fruchtansatz aufwies.

c) Die Nachkommenschaft der selbstbestäubten, selbststerilen Pflanze FII 3 und Interpretation der Kreuzungsresultate. Gleichzeitig mit der Untersuchung der Nachkommenschaft der Kreuzung FII 13 × FII 3 auf ihre Kreuzungsfertilität bzw. -sterilität wurden 13 Nachkommen der selbststerilen Pflanze FII 3 selbstbestäubt und reziprok in allen möglichen Kombinationen miteinander gekreuzt. Die Samen waren durch die bei den physiologischen Experimenten beschriebenen Methoden der Selbstbestäubung (vgl. S. 373) erhalten worden (Tabelle 4).

Tabelle 4. *Resultate der Selbst- und Kreuzbestäubungen in der Nachkommenschaft der Selbstung von F II 3, verbunden mit einer Bestimmung der genetischen Konstitution der 3 Gruppen A, B und C*

Gruppe	Konstitution	Pflanzen Nr. G_1 3/	A							B					C
			S_1S_4							S_1S_1					S_4S_4
			Z_2Z_2	Z_2Z_3	Z_3Z_3					Z_2Z_2	Z_2Z_3	Z_3Z_3			Z_2Z_2 Z_2Z_3 Z_3Z_3
			3	6	8	12	13	17	19	4	5	7	9	11	15
A	S_1S_1	3	-						?		-		-	-	—
	Z_2Z_2	6													
	Z_2Z_3	8	—	—					--		-			-	
	Z_3Z_3	12												-	
		13	-												
		17													
		19	-	—	-	—	—		—	-		--	—	—	-
B	S_1S_1	4	--	-				-	-		—	-		-	+
	Z_2Z_2	5							-		-			-	---
	Z_2Z_3	7							-			—	-	—	---
	Z_3Z_3	9		--	-	—			-	-		—	-	-	--
		11	—	—		---	—	—	—	—	-		—		+
C	S_1S_4	15	-							---					—
	Z_2Z_2														
	Z_2Z_3														
	Z_3Z_3														

Sämtliche 13 Pflanzen waren selbststeril. 1 Pflanze G_1 3/15 setzte, als Mutter verwendet, mit allen übrigen 12 Pflanzen an; als Pollenspender verwendet, konnte sie jedoch nur auf 5 Pflanzen einen Fruchtansatz erzielen. Abgesehen von den Bestäubungen, an denen die Pflanze G_1 3/15 beteiligt war, fielen sämtliche Kreuzungsbestäubungen innerhalb der 12 Pflanzen bis auf eine Ausnahme negativ aus. Die Ausnahme bildete die Kreuzung G_1 3/3 × G_1 3/19, bei der von 4 Bestäubungen 2 angesetzt hatten. Bei 21 von 169 Kreuzungen waren die Resultate nicht eindeutig. Sie wurden wie in Tabelle 3 ausgewertet.

Da die selbstbestäubte Pflanze F II 3 die genetische Konstitution $S_1S_4Z_2Z_3$ besaß, konnten ihre Nachkommen nur die in Tabelle 4 aufgeführten Genkombinationen enthalten. Die Kreuzungsresultate machten eine Einteilung der Nachkommenschaft in 3 Gruppen A, B und C notwendig. Die 3 Gruppen unterscheiden sich durch ihre *S*-Allele. Die Gruppe A enthält die Allele S_1S_4, die Gruppe B die Allele S_1S_1 und die eine Pflanze G_1 3/15 der Gruppe C die Allele S_4S_4. Da sich die Pflanzen innerhalb einer jeden Gruppe in bezug auf ihre Kreuzungsresultate einheitlich verhalten, können die 3 möglichen Kombinationen der Z-Allele Z_2Z_2, Z_2Z_3 und Z_3Z_3 beliebig auf die einzelnen Pflanzen der 3 Gruppen verteilt werden. Die Kreuzungsresultate lassen sich also in diesem Fall unabhängig von der Wirkung der *Z*-Allelenserie erklären. Eine Epistasie der *Z*-Allele über die *S*-Allele ist unter den gegebenen Genkombinationen nicht möglich.

Die in Tabelle 4 aufgeführten Kreuzungsresultate sind ein weiterer Beweis dafür, daß die Voraussetzung der sporophytischen Determination des Pollens zu Recht besteht. Bei gametophytischer Determination hätte die Gruppe B mit der Gruppe A ansetzen müssen, da der Pollen der Gruppe A zur Hälfte das Allel S_4 enthalten mußte, welches in der Gruppe B nicht vorhanden ist.

2. Genetik der erblichen Pseudofertilität. Unabhängig von dem Alter der Pflanzen und dem Alter der Blüten traten unter den auf Selbststerilität hin geprüften Pflanzen zahlreiche Individuen auf, die einen Ansatz nach Selbstbestäubung aufwiesen. Die Ansatzprozentsätze schwankten unter diesen Pflanzen sehr stark. Sie bewegten sich zwischen 8 und 73%. Einen 100%igen Fruchtansatz zeigte keine Pflanze. Die Samenzahlen in den durch Selbstbestäubung erzielten Früchten waren im allgemeinen sehr reduziert. Das Auftreten dieser mehr oder weniger stark pseudo-selbstfertilen Pflanzen führte zu dem Versuch, das Verhältnis von selbststerilen zu pseudo-selbstfertilen Individuen in 5 Nachkommenschaften verschiedenen Ursprungs zahlenmäßig zu erfassen. Dieser Versuch mußte zeigen, ob die beobachtete Pseudo-Selbstfertilität genetisch oder wie Knospen- und Alters-Pseudofertilität phänotypisch bedingt ist. Als pseudo-selbstfertil wurden alle die Pflanzen bewertet, bei denen mehr als 25% der Bestäubungen positiv ausgefallen sind; alle restlichen Pflanzen wurden als selbststeril angesehen.

Tabelle 5. *Verhältnis von selbststerilen (sst): pseudo-selbstfertilen (psf) Pflanzen in den Nachkommenschaften von 3 Fremdungen und 2 Selbstungen*

Fremdung / Selbstung	sst psf	sst psf
E/4 (sst) × E/5 (psf)	10 : 8 =	1,25 : 1
E/5 (psf) × E/4 (sst)	4 : 3 =	1,33 : 1
F II 13 (sst) × F II 3 (sst)	15 : 1 =	15,0 : 1
F II 3 (sst) × selbst.	15 : 0 =	15,0 : 0
F II 4 (psf) × selbst.	6 : 4 =	1,5 : 1

a) Aufführung der Spaltungsverhältnisse von selbststerilen (sst): pseudo-selbstfertilen (psf) Individuen in den Nachkommenschaften von Selbstungen und Fremdungen (Tabelle 5). Das Auftreten dieser Spaltungszahlen und das Ausbleiben pseudo-selbstfertiler Individuen unter den Nachkommen der Kreuzungen, an denen selbststerile Pflanzen beteiligt sind (die pseudo-selbstfertile Pflanze aus Kreuzung F II 13 × F II 3 soll unberücksichtigt bleiben), sprechen für eine genetische Determination dieser Pseudofertilität. Bei einer phänotypisch bedingten Pseudofertilität hätten unter den Nachkommen der selbststerilen Pflanze F II 3 und der Kreuzung F II 13 × F II 3 wenigstens einige Pflanzen Pseudo-Selbstfertilität zeigen müssen.

b) Genetische Interpretation der Spaltungsverhältnisse mit Hilfe einer Arbeitshypothese. Trotz der Kleinheit der Spaltungszahlen scheint es gerechtfertigt, die aufgefundenen Spaltungsverhältnisse genetisch zu deuten. Unsere Arbeitshypothese basiert auf folgenden Voraussetzungen: Neben den beiden hemmenden *S*- und *Z*-Allelenserien ist ein weiteres Allelenpaar $F_1 F_2$ vorhanden, das *in heterozygotem Zustand eine mehr oder minder starke Aufhebung der von den S- und Z-Allelen nach Selbstung hervorgerufenen Hemmung bedingt.* Liegen die *F*-Allele in einer Pflanze neben den *S*- und *Z-Allelen* in homozygoter Form vor, dann überwiegt die Wirkung der hemmenden Allele. Wie man sich die gegenseitige Beeinflussung der beiden *F*-Allele und die Wechselwirkung zwischen dem heterozygot vorliegenden *F*-Allelenpaar und den beiden Hemmserien vorstellen kann, soll später erörtert werden.

Mit Hilfe dieser Hypothese lassen sich die in Tabelle 5 aufgeführten Spaltungsverhältnisse erklären. Betrachten wir zunächst die beiden Kreuzungen E/4 × E/5 und E/5 × E/4. Als pseudo-selbstfertiles Individuum muß die Pflanze E/5 neben

den *S*- und *Z*-Allelen die *F*-Allele in heterozygoter Form enthalten. Die selbststerile Pflanze E/4 muß die Konstitution $S_nS_nZ_nZ_nF_1F_1$ oder $S_nS_nZ_nZ_nF_2F_2$ besitzen. Die Nachkommen von E/4 × E/5 und E/5 × E/4 müssen also zur Hälfte selbststeril, zur Hälfte pseudo-selbstfertil sein (heterozygot × homozygot).

Die Nachkommenschaft einer Kreuzung, an der ausschließlich selbststerile Pflanzen (FII 13 × FII 3) beteiligt sind, kann entsprechend der Konstitution der Elternpflanzen ($S_nS_nZ_nZ_nF_1F_1$ oder $S_nS_nZ_nZ_nF_2F_2$) entweder nur aus pseudo-selbstfertilen ($F_1F_1 \times F_2F_2$) oder nur aus selbststerilen Individuen ($F_1F_1 \times F_1F_1$) bestehen. In vorliegendem Fall dürfte es sich um die Kreuzung zweier Formen mit gleichen *F*-Allelen gehandelt haben. Auch der Befund, wonach die Nachkommen der selbstbestäubten, selbststerilen Pflanze FII 3 sämtlich selbststeril sind, ist auf Grund der angenommenen Homozygotie in den *F*-Allelen der selbststerilen Pflanze leicht zu deuten. Schließlich ist die Selbstung der pseudo-selbstfertilen Pflanze FII 4 zu interpretieren. Sie spaltet in ihrer Nachkommenschaft in 1.5 Teile selbststeriler und 1 Teil pseudo-selbstfertiler Individuen auf. Gemäß der aufgestellten Hypothese müßte diese Selbstung zur Hälfte selbststerile (*F*-homozygote), zur Hälfte pseudo-selbstfertile (*F*-heterozygote) Individuen ergeben. Das tatsächlich gefundene Verhältnis weicht also von dem theoretischen ab, doch muß diese Abweichung nicht gegen die Gültigkeit der aufgestellten Hypothese von der Wirksamkeit der *F*-Allele sprechen.

II. Physiologische Untersuchungen

1. Pollenkeimung und Pollenschlauchwachstum in vivo. Zur Untersuchung der Pollenschlauchkeimung und des Pollenschlauchwachstums wurden Griffellängsschnitte mit dem Mikrotom hergestellt. Die Anfärbung der Schnitte erfolgte nach der von Huber und Schoch-Bodmer (1944) abgeänderten Heidenhainschen Azanfärbetechnik. Aus den Präparaten war folgendes zu ersehen: Die Wachstumsgeschwindigkeit von Fremdschläuchen[1] liegt bei 0,9 mm/h. Die Samenanlagen werden in jedem Fall nach 8 Std erreicht. Die Selbstschläuche sind in ihrem Wachstum extrem stark gehemmt; sie werden meistens schon kurz nach ihrem Auskeimen auf der Narbe gestoppt. Es war zu klären, ob nur das Schlauchwachstum gehemmt wird, oder ob schon die Keimungsrate des Pollens eine Reduktion erfährt. Hierzu wurden selbststerile *R. raphanistrum*-Pflanzen fremd- und selbstbestäubt und 2, 8, 12 und 24 Std nach erfolgter Bestäubung Karminessigsäure-Quetschpräparate der bestäubten Narben hergestellt. In den Präparaten wurde die Anzahl der gekeimten Pollenkörner auf den Narben bestimmt. Als gekeimt galten Körner, deren Schläuche länger als $^1/_2$ Pollenkorndurchmesser waren. Die Ergebnisse dieser Auszählungen sind in Tabelle 6 zusammengefaßt.

Tabelle 6. *Keimprozente des Pollens in vivo n-Stunden nach Fremd- und Selbstbestäubung*

Art der Bestäubung	Anzahl der Narben	n	Gekeimt	Nicht gekeimt	Total	%
Fremdung	10	2	1874	2694	4568	41,0
Selbstung	10	2	307	3875	4182	7,3
Selbstung	6	8	501	2997	3498	14,3
Selbstung	6	12	869	2420	3289	26,4
Selbstung	7	24	1098	3141	4239	25,9

[1] Mit „Fremdschläuchen" werden Schläuche bezeichnet, die aus einer Fremdbestäubung hervorgehen. Das entsprechende gilt für „Selbstschläuche".

Beim Vergleich der Keimprozente in Tabelle 6 zeigt sich, daß nach 2stündiger Keimdauer ein deutlicher Unterschied zwischen Selbst- und Fremdbestäubung besteht. Ferner ersieht man aus den Werten, daß die Pollenkeimung zum Teil gehemmt ist. 24 Std nach Selbstbestäubung konnte auf den Narben nur ein durchschnittlicher Keimprozentsatz von 25,9 ermittelt werden. Da dieser Wert gegenüber dem Keimprozentsatz nach 12stündiger Keimdauer nicht erhöht ist, kann angenommen werden, daß die Keimprozente nach längerer Keimdauer nicht weiter ansteigen.

2. Versuche zur Lokalisierung der Hemmung. Nachdem festgestellt worden war, daß das Schlauchwachstum nach Selbstbestäubung schon auf der Narbe gestoppt wird, mußte geprüft werden, ob sich die Hemmung wie bei *Brassica oleracea* (Sears 1937) und *Raphanus sativus* (Tatebe 1939) nur auf die Narbenoberfläche erstreckt, oder ob auch das tiefer liegende Narbengewebe und der Griffel einen Hemmeffekt auf Pollenkeimung und Schlauchwachstum ausüben. Zur Klärung dieser Frage wurden Selbstbestäubungen nach 5 verschiedenen Bestäubungsmethoden durchgeführt. Sie sind in Abb. 1 dargestellt. Die Resultate bringt Tabelle 7.

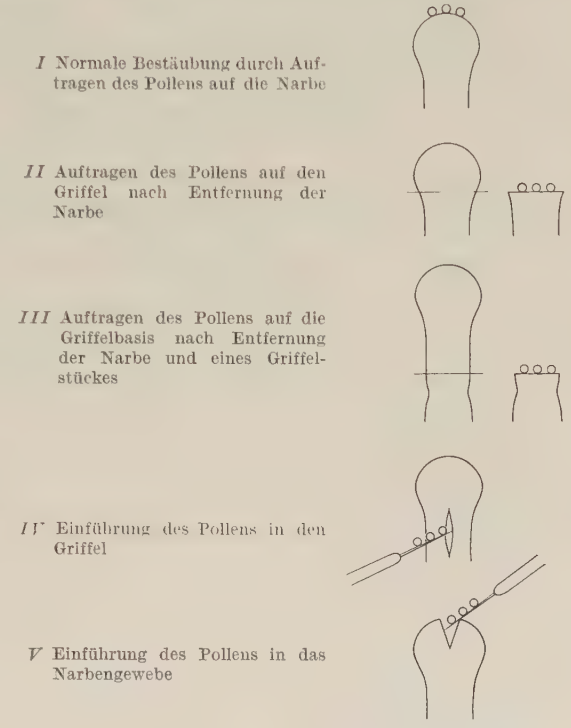
I Normale Bestäubung durch Auftragen des Pollens auf die Narbe
II Auftragen des Pollens auf den Griffel nach Entfernung der Narbe
III Auftragen des Pollens auf die Griffelbasis nach Entfernung der Narbe und eines Griffelstückes
IV Einführung des Pollens in den Griffel
V Einführung des Pollens in das Narbengewebe

Abb. 1. Darstellung von verschiedenen Bestäubungsmethoden

Tabelle 7 läßt erkennen, daß die nach den Methoden II—V bestäubten streng selbststerilen Klone einen Fruchtansatz zeigten, dessen Höhe von der Bestäubungs-

Tabelle 7. *Selbstbestäubungen nach den Methoden I—V*

Nr. der Klone	Methode	Anzahl der bestäubten Blüten	Angesetzt	Nicht angesetzt	Fruchtanstz %	
F II 3	I	95	1	94	1,0	
F II 13	I	74	0	0	0,0	
G 3/1	I	38	0	0	0,0	
F II 3	II	73	16	57	21,9	$>\chi^2 = 0{,}052$ N = 1, P = 0,90—0,80
F II 13	II	29	7	22	24,1	
F II 3	III	37	8	29	21,6	$>\chi^2 = 0{,}011$ N = 1, P = 0,90—0,80
F II 13	III	13	3	10	23,0	
F II 3	IV	54	25	29	46,2	$>\chi^2 = 1{,}297$ N = 1, P = 0,30—0,20
F II 13	IV	56	32	24	57,1	
G 3/1	V	35	28	7	80,0	

N = Anzahl der Freiheitsgrade.

methode abhängig ist. Aus den P-Werten geht hervor, daß sich die beiden Klone FII 3 und FII 13 in ihren Fruchtansätzen innerhalb der einzelnen Bestäubungsmethoden nicht voneinander unterscheiden. Ebenso differieren die Methoden II und III in ihrem Erfolg nicht (Tabelle 8).

Tabelle 8. *Sicherung der Differenzen in den Tabellen 7 und 9 nach der χ^2-Methode*

Nr. der Tabelle	Methode	χ^2	N	P
7	II/III	0,017	1	0,90—0,80
7	II/IV	19,308	1	$<0{,}001$
7	IV/V	8,688	1	0,01—0,001
9	II/IV	7,483	1	0,01—0,001
7/9	II	0,033	1	0,90—0,80
7/9	IV	0,210	1	0,70—0,50

Die durch Anwendung der Methoden II und III gewonnenen Ergebnisse beweisen, daß es gleichgültig ist, auf welcher Griffelhöhe die Bestäubung erfolgt. Im Griffel kann also kein Hemmungsgefälle bestehen.

Um festzustellen, inwieweit die Höhe der durch die Methoden II—V erzielten Fruchtansätze von dem technischen Eingriff als solchem abhängig ist, wurden Fremdbestäubungen auch nach den Methoden II und IV durchgeführt. Aus Tabelle 9 ist ersichtlich, daß nach Methode I durchgeführte Fremdungen einen 100%igen Fruchtansatz zeigten. Bei den entsprechenden nach Methode II und IV vollzogenen Fremdbestäubungen war der Fruchtansatz jedoch stark reduziert, nämlich auf 22,3% nach Methode II und auf 45,9% nach Methode IV. Vergleicht man diese beiden Werte mit den entsprechenden der Selbstbestäubungen in Tabelle 7, dann findet man zwischen ihnen eine gute Übereinstimmung. (Ihre Differenzen sind nach der χ^2-Methode nicht gesichert; Tabelle 8). Der technische Eingriff der Bestäubungsmethoden II—V spielt also bei den Ansatzprozentsätzen eine erhebliche Rolle. Die Vermutung liegt nahe, daß die unter Umgehung der hemmenden Narbenoberfläche vollzogenen Bestäubungen einen 100%igen Fruchtansatz ergeben haben würden, wenn er nicht durch den Eingriff als solchen reduziert worden wäre.

Tabelle 9. *Fremdbestäubungen nach den Methoden I, II und IV*

Kreuzung	Methode	Anzahl der bestäubten Blüten	Angesetzt	Nicht angesetzt	Fruchtansatz %
FII 8 × FII 24 FII 24 × FII 8	I	36	36	0	100
FI 5 × FI 32 FI 24 × FII 1 FI 29 × FII 1 FII 4 × FI 21 FII 4 × FI 27	I	22	22	0	100
FII 8 × FII 24 FII 24 × FII 8	IV	63	28	35	45,9
FI 5 × FI 32 FI 24 × FII 1 FI 29 × FII 1 FII 4 × FI 21 FII 4 × FI 27	II	61	13	48	22,3

Tabelle 10. *Mittlere Samenzahl in Früchten verschiedener Herkunft*

Herkunft der Früchte	Anzahl der Früchte	Anzahl der Samen	M ± m
FII 3 × selbst	45	165	3,7 0,276
FII 13 × selbst	32	132	4,1 0,323
FII 8 × FII 24 FII 24 × FII 8	36	144	4,0 0,416

Ergänzend wurde geprüft, ob zwischen den durch Selbstbestäubung nach den verschiedenen Methoden erzielten Früchten und den durch Fremdbestäubung gewonnenen ein Unterschied hinsichtlich ihrer Samenanzahl bestand. Es wurden hierzu die Anzahl der Samen von 113 Früchten, die teils aus Selbstungen, teils aus Fremdungen hervorgegangen waren, bestimmt. Sie sind in Tabelle 10 aufgeführt. Ihre Mittelwerte unterscheiden sich nicht (Tabelle 11).

Tabelle 11. *Sicherung der Differenzen in Tabelle 10 nach dem t-Test*

Herkunft der Früchte	d	sd	t	N	P
F II 3/F II 13	0,430	0,425	1,01	76	0,40—0,30
F II 3/F II 8 u. 24	0,340	0,499	0,68	79	0,50 –0,40
F II 13/F II 8 u. 24	0,090	0,466	0,19	67	0,90—0,80

d = Differenz der Mittelwerte, sd = Streuung der Differenz, N = Anzahl der Freiheitsgrade.

3. Pseudofertilitätsuntersuchungen. *a) Knospen-Pseudofertilität.* In diesen Versuchen soll die Abhängigkeit des Fruchtansatzes von der Länge der bestäubten Knospen bestimmt werden. Zu den Bestäubungen diente der selbststerile Klon G3/1, der 8 Pflanzen umfaßte.

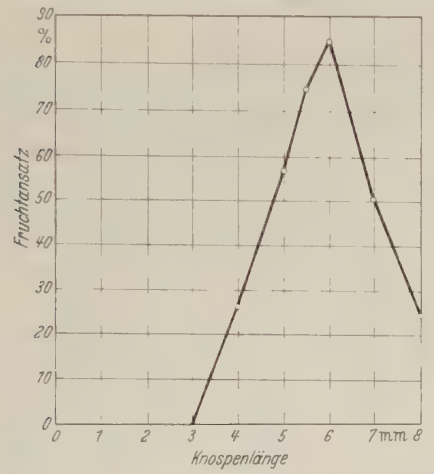

Abb. 2.

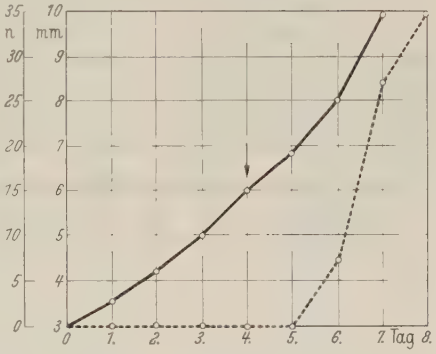

Abb. 3.

Abb. 2. Abhängigkeit des Fruchtansatzes von der Länge der bestäubten Knospen

Abb. 3. Zeitliche Abhängigkeit des Knospenwachstums und der Knospenöffnung. Der Pfeil weist auf den Punkt hin, wo die für eine Bestäubung günstigste Knospenlänge vorliegt. o——o Knospenwachstum; o- - -o Knospenöffnung; *n* Anzahl der geöffneten Knospen

Die Ergebnisse der Knospenbestäubungen sind in Abb. 2 graphisch dargestellt. Es liegt eine deutliche Pseudofertilität vor. Den höchsten Fruchtansatz mit 83,3% zeigten 6 mm lange Knospen.

Um die Ergebnisse der Knospenbestäubungsversuche von *R. raphanistrum* mit denjenigen anderer Cruciferen vergleichen zu können, bei denen als Kriterium des Ansetzens die Zeit in Tagen vor der Knospenöffnung angegeben ist, mußte festgestellt werden, nach wieviel Tagen 6 mm lange Knospen aufblühen. Hierzu wurde einmal das Längenwachstum und zum anderen die zeitliche Folge der Öffnung von insgesamt 35 Knospen bestimmt (Abb. 3).

Die Ausgangsgröße der Knospen betrug 3 mm. Am 4. Tag nach Beginn der Messung haben sie die für den Erfolg der Bestäubung günstigste Länge von 6 mm erreicht. Da am 7. Tag nach Versuchsbeginn die Mehrzahl der Knospen aufgehen,

kann als günstigste Zeit für eine erfolgreiche Bestäubung der 3. Tag vor der Knospenöffnung angegeben werden.

b) Pseudofertilität alternder Blüten. Zur Feststellung, ob nach Selbstbestäubung älterer Blüten Fruchtansatz auftritt, wurden Blüten der selbststerilen G4/1-Pflanze 1, 2 und 3 Tage nach ihrer Öffnung selbstbestäubt. Zu den Bestäubungen wurde Pollen von frisch geöffneten Blüten derselben Pflanze verwandt. Wie Tabelle 12 zeigt, setzte in jeder der drei Bestäubungsserien eine Frucht an. Da die drei Früchte aus 48 bestäubten Blüten nicht mehr als 1 bzw. 2 Samen enthielten, konnte man nicht von einer Pseudofertilität sprechen.

Tabelle 12. *Selbstbestäubung verschieden alter Blüten der Pflanze G 4/1*

Alter der Blüten in Tagen	Anzahl der bestäubten Blüten	Angesetzt	Nicht angesetzt	Anzahl der Samen
1	14	1	13	1
2	14	1	13	1
3	20	1	19	2

c) Alters-Pseudofertilität („end-season-fertility"). Zu diesem Versuch wurden drei Pflanzen des selbststerilen Klons G 3/4 verwendet. Zu Beginn der Vegetationsperiode wurden an den Pflanzen 30 Selbstbestäubungen vorgenommen, die sämtlich negativ ausfielen. Gegen Ende der Blühperiode wurden abermals Selbstbestäubungen ausgeführt. Hierbei setzten von 55 bestäubten Blüten nur 2 Blüten Früchte mit 2 und 1 Samen an. Das Bestäubungsresultat spricht gegen eine Alterspseudofertilität.

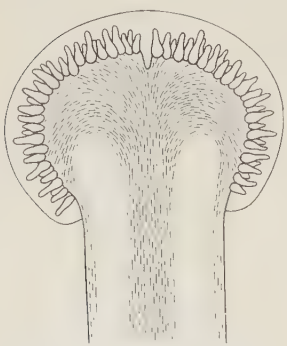

Abb. 4. Narbe mit Gelatineüberzug im Längsschnitt

4. Pollenkeimung in vitro. Bevor mit dem Versuch begonnen werden konnte, die auf der Narbe nach Selbstbestäubung stattfindende Hemmungsreaktion *in vitro* zu reproduzieren, war es notwendig, die optimalen Keimungsbedingungen für *R. raphanistrum*-Pollen aufzufinden.

Methodik. Zu den Keimungsversuchen wurde stets spontan ausstäubender Pollen verwendet. Die Kulturen verwahrten wir bei einer konstanten Temperatur von 21° C ± 1°. Als gekeimt galten Pollenkörner, deren Schlauchlänge > 1,5 Pollenkorndurchmesser war. Es wurden stets die Gesichtsfelder ausgezählt, in denen sich die meisten gekeimten Pollenkörner befanden.

Zahlreiche Versuchsreihen führten zu dem Ergebnis, daß *R. raphanistrum*-Pollen nur mit Hilfe der Methode der „indirekten Wasseraufnahme" zum Keimen gebracht werden kann. Die Keimung vollzieht sich bei diesem Verfahren auf einem anfangs trockenen, in einem feuchten Raum quellenden Substrat. Als bestes Keimsubstrat erwies sich für *R. raphanistrum*-Pollen 5- und 10%ige Gelatine, auf der im Mittel 50% Körner auskeimten.

5. Pollenkeimung auf „Narbengelatine". In dem folgenden Versuch kam es darauf an, das sich auf der Narbe auswirkende hemmende Prinzip auf das Keimmedium zu übertragen. Das Keimmedium sollte daher möglichst keine Zusätze enthalten, da diese unter Umständen unkontrollierbare Verbindungen mit dem Hemmprinzip eingehen konnten. Die Gelatinelösung erfüllte diese Forderung.

Methodik. Die zwei verwendeten selbststerilen Klone, die miteinander kreuzungsfertil waren, seien der Einfachheit halber mit A und B bezeichnet. Zu jedem Versuch dienten je 4 Blüten von Klon A und B. Die Blüten wurden mit dem Stiel von der Pflanze abgelöst, Blüten- und Kelchblätter auf die Hälfte reduziert und nach A und B getrennt in kleine, mit Wasser gefüllte Glasröhrchen gesetzt. Anschließend überzogen wir die Narben der Blüten mit einem 10%igen Gelatinetropfen (Abb. 4). Um ein Erstarren der Gelatine auf der Narbe zu verhindern, mußten die Glasröhrchen mit den Blüten in einem mit feuchtem Filtrierpapier ausgeschlagenen Glasgefäß gehalten werden, das wir in einem Thermostaten von 21° C verwahrten. Nach 24 Std wurde die Gelatineschicht von den 8 Narben abgelöst und nach A und B getrennt auf je einem Deckglas ausgestrichen. In dieser Zeitspanne konnten Substanzen, die bei der Hemmreaktion nach Selbstung eine Rolle spielen, wenn sie überhaupt diffusibel sind, aus den Narbenpapillen in die Gelatine übergetreten sein. Auf die Gelatineschicht der beiden Deckgläser wurde Pollen von Klon A oder Klon B gestäubt und die Deckgläser auf Hohlschliffobjektträger aufgesetzt. Die Keimung des Pollens erfolgte über einem 25%igen Zuckerlösungstropfen. Als gekeimt galten in dieser Versuchsserie Pollenkörner, deren Schlauchlänge nur $^1/_2$ Pollenkorndurchmesser betrug. Es war notwendig, dieses geringe Maß als Kriterium der Keimung festzusetzen, da das Schlauchwachstum ganz allgemein auf der „Narbengelatine" sehr reduziert war.

Tabelle 13. *Keimprozente des Pollens auf „Narbengelatine"*

Nr. des Versuchs	Selbstung				Fremdung			
	gekeimt	nicht gekeimt	total	%	gekeimt	nicht gekeimt	total	%
1	71	147	218	32,5	78	142	220	35,5
2	37	91	128	28,8	24	140	164	14,6
3	34	115	149	22,8	35	91	126	27,8
4	46	147	193	23,8	95	96	191	49,7
5	75	147	222	34,0	92	136	228	40,3
6	92	142	234	39,3	104	147	251	41,4
7	5	145	150	3,3	76	119	195	38,9
8	29	93	122	23,7	39	83	122	31,9
9	62	140	202	30,6	69	144	213	32,3
10	81	133	214	37,8	33	115	148	22,2
11	30	231	261	11,4	38	107	145	26,2
12	21	147	168	12,5	74	127	201	36,8
13	15	139	154	9,7	43	158	201	21,3
14	7	143	150	4,6	49	146	195	25,1
15	0	150	150	0,0	26	139	165	15,7
16	66	157	223	29,5	53	103	156	33,9
17	38	168	206	18,4	16	148	164	9,7
18	75	123	198	37,8	44	161	205	21,4
19	54	153	207	26,0	57	141	198	28,7
20	56	149	205	27,3	77	124	201	38,3
21	68	137	205	33,1	57	146	203	28,0
22	40	166	206	19,4	45	156	201	22,3
23	71	137	208	34,1	96	118	214	44,8
24	65	145	210	30,9	73	136	209	34,9
25	76	130	206	36,8	92	126	218	42,2
Total	1214	3575	4789	25,3	1485	3249	4734	31,3

$\chi^2 = 42{,}44,\ N = 1,\ P = < 0{,}001.$

Die Ergebnisse dieser Versuchsreihe sind in Tabelle 13 wiedergegeben. Vergleicht man die Keimprozente des Pollens nach einer „Selbstung" mit den Keimprozenten nach einer „Fremdung", dann stellt man fest, daß sie in 20 von 25 Fällen bei der „Fremdung" höher liegen als bei der „Selbstung". Wenn die Unterschiede zwischen den entsprechenden Keimprozentsätzen auch oft nur gering sind, so sind unter Berücksichtigung der gesamten 25 Versuche die Differenzen zwischen

den „Selbstungs"- und „Fremdungs"-Werten mit einem P-Wert von $< 0{,}001$ sehr gut gesichert.

Aus diesem Versuchsresultat kann geschlossen werden, daß die in der Narbenoberfläche nach Selbstbestäubung in Aktion tretenden „hemmenden Substanzen" wenigstens zum Teil in die „Narbengelatine" übergetreten sein müssen.

D. Besprechung der Ergebnisse

I. Die Selbst- und Kreuzungssterilität

Die beschriebenen Kreuzungsresultate in der Nachkommenschaft der Kreuzung FII 13 × FII 3 (Tabelle 3) zeigen auffallend häufig reziproke Unterschiede. Unterschiedliche Ergebnisse bei reziproken Kreuzungen sind unter den selbststerilen Pflanzen weit verbreitet und werden u. a. von Correns (1913), Stout (1917), East und Mangelsdorf (1925), Kakizaki (1930), Tatebe (1940) und Hughes und Babcock (1950) erwähnt. Jede genetische Theorie muß daher dieser Erscheinung Rechnung tragen. Dies kann auf verschiedene Weisen geschehen.

1. Nach dem Eastschen *Nicotiana*-Schema lassen sich reziproke Unterschiede bei einer Kreuzung dadurch erklären, daß eine homozygote Pflanze an der Kreuzung beteiligt ist.

2. Das für die Compositen charakteristische Auftreten reziproker Unterschiede bei Kreuzungen selbststeriler Pflanzen wurde von Gerstel (1950) für *Parthenium argentatum*, Hughes und Babcock (1950) für *Crepis foetida* und Crowe (1954) für *Cosmos bipinnatus* durch die Annahme sporophytischer Determination des Pollens und des Auftretens von Dominanzverhältnissen zwischen den S-Allelen erklärt.

3. Die reziproken Unterschiede, die sowohl bei den Kreuzungen selbststeriler mit selbststerilen als auch bei den Kreuzungen zwischen selbststerilen und pseudoselbstfertilen *Brassica oleracea*-Pflanzen auftraten, wurden von Kakizaki (1930) mit Hilfe der Annahme von 2 Allelenserien gedeutet, von denen die eine einen hemmenden, die andere einen fördernden Einfluß auf das Pollenschlauchwachstum ausübt. Die Annahme eines solchen Assoziationstyps war erforderlich, da die Kreuzungsexperimente an *Brassica oleracea* u. a. noch folgende Resultate aufwiesen:

a) Auftreten von mehr als 4 Genotypen in der Nachkommenschaft von zwei Pflanzen einer gegebenen genetischen Konstitution,

b) Auftreten pseudo-selbstfertiler Pflanzen unter den Nachkommen gewisser selbststeriler Genotypen.

Auch für *R. raphanistrum* ist die Annahme von mehr als einer Allelenserie berechtigt, denn die zahlreichen reziproken Unterschiede lassen sich nur auf Grund des Auftretens von mehr als 4 Genotypen in der Nachkommenschaft erklären. Da sie in einer Nachkommenschaft auftraten, unter der sich kein pseudo-selbstfertiles Individuum befand, mußte entgegen der Kakizakischen Hypothese zunächst das Vorhandensein zweier hemmender Allelenserien (S und Z) angenommen werden, von denen die S-Serie im allgemeinen epistatisch über die Z-Serie war.

Wie bei *Crepis foetida* (Hughes und Babock 1950) und *Parthenium argentatum* (Gerstel 1950) müssen die Pollenkörner bei *R. raphanistrum* sporophytisch deter-

miniert sein und innerhalb der Allelenserien im Pollen Dominanzverhältnisse auftreten.

Zur Erklärung der Kreuzungsresultate in Tabelle 3 war noch folgende Annahme notwendig: Unter der Voraussetzung, daß in einer Kreuzung das im Pollen rezessive *S*-Allel auch im Griffel vorhanden ist, beispielsweise in der Kreuzung $S_3S_4Z_1Z_2 \times S_2S_4Z_1Z_2$ das Allel S_4, vermögen die *Z*-Allele die Reaktion zwischen Pollen und Narbe zu bestimmen. In einer derartigen Kreuzung können sie einen Fruchtansatz, der auf Grund des dominanten Allels im Pollen stattfinden könnte, verhindern, wenn wir annehmen, daß in einem solchen Fall bestimmte *Z*-Allele epistatisch über bestimmte *S*-Allele sind. Da die Epistasieverhältnisse *nicht* auftreten, wenn *Pollen und Griffel sich in beiden S-Allelen* unterscheiden [Kreuzung: Gruppe A ($S_3S_4Z_nZ_n$) × Gruppe B ($S_1S_2Z_nZ_n$) und B × A], muß dem rezessiven *S*-Allel im Pollen doch noch eine gewisse Wirkung zugesprochen werden. Diese kann man sich vielleicht so vorstellen, daß in der Kreuzung $S_3S_4Z_nZ_n \times S_1S_2Z_nZ_n$ das rezessive S_2-Allel die das Schlauchwachstum begünstigende Wirkung des S_1-Allels noch zusätzlich fördert, während in der Kreuzung $S_3S_4Z_nZ_n \times S_2S_4Z_nZ_n$ das rezessive S_4-Allel unter der Einwirkung des identischen Griffel-Allels die Wirkung des dominanten S_2-Allels abschwächt, so daß sich die Gleichheit der *Z*-Allele in Form einer Hemmung auswirken mag.

Vergleichen wir die für *R. raphanistrum* aufgestellte genetische Hypothese mit den genetischen Schemata, die für die Cruciferenarten *Brassica oleracea*, *Capsella grandiflora*, *Cardamine pratensis* und *Raphanus sativus* entwickelt worden sind, dann kann festgestellt werden, daß sie sich am meisten dem Schema Tatebes (1944) für *Raphanus sativus* annähert. Abgesehen von Einzelheiten (Anzahl der Allele in einer Serie, Epistasieverhältnisse), unterscheidet sich unsere Hypothese von der Tatebes nur durch die Annahme von Dominanzverhältnissen zwischen den Allelen der beiden Serien.

II. Die erbliche Pseudofertilität

Es bestehen zwei verschiedene Hypothesen zur Erklärung der erblichen Bedingtheit des Fruchtansatzes nach Selbstbestäubung „selbststeriler" Pflanzen. Die Experimente von Compton (1912) an *Reseda odorata*, Baur (1919) an *Antirrhinum*, East (1919) und Brieger (1927) an *Nicotiana* und Riley (1936) an *Capsella grandiflora* führten zu der Annahme eines dominanten Fertilitätsfaktors, der von East und Yarnell (1929) als ein Glied der *S*-Allelenreihe angesehen und mit S_f bezeichnet wurde. Die Versuchsergebnisse an *Brassica oleracea* und *Cardamine pratensis* veranlaßten Kakizaki (1930) und Beatus (1934) zu der Hypothese einer zweiten, das Pollenschlauchwachstum fördernden Allelenserie.

Zur Deutung der in Tabelle 5 aufgeführten Spaltungsverhältnisse können diese beiden Hypothesen nicht herangezogen werden. Die erste Hypothese muß abgelehnt werden, weil die Nachkommenschaft der selbstbestäubten, pseudo-selbstfertilen Pflanze FII 4 nicht in einem 3 psf : 1 sst-Verhältnis aufspaltet, das für die Anwesenheit eines dominanten Fertilitätsfaktors in dieser Pflanze gesprochen hätte. Die Zahl der selbststerilen Pflanzen ist vielmehr etwas größer als die der pseudo-selbstfertilen Individuen. Auch mit Hilfe der von Kakizaki und Beatus aufgestellten Hypothese können die Spaltungsverhältnisse in Tabelle 5 nicht erklärt werden. Im Gegensatz zu den Befunden bei *Brassica oleracea* und

Cardamine pratensis traten nämlich in unserem Material in einer Nachkommenschaft *nur pseudo-selbstfertile Individuen auf, wenn pseudo-selbstfertile Pflanzen an der betreffenden Kreuzung bzw. Selbstung beteiligt waren.* Zur Deutung der Spaltungszahlen wurde daher ein weiteres Faktorenpaar F_1F_2 herangezogen, dessen Wirkung man sich folgendermaßen vorstellen könnte: F_1 und F_2 sind keine Fertilitätsfaktoren, sondern sie schwächen, wenn sie in heterozygoter Form vorliegen, die das Schlauchwachstum hemmende Wirkung der *S*- und *Z*-Allele ab, so daß es zu einem Fruchtansatz kommen kann. F_1 und F_2 spielen also die Rolle von Modifikationsfaktoren, die nicht direkt, wie die von Kakizaki (1930) angenommene Förderungsserie, sondern indirekt über die *S*- bzw. *Z*-Allele auf das Pollenschlauchwachstum einwirken. Je nach der Konstellation der *S*- und *Z*-Allele kann das heterozygote *F*-Faktorenpaar die beiden Hemmserien verschieden stark beeinflussen, so daß ein wechselnder Fruchtansatz erzielt wird.

Für diese Deutung sprechen zwei Befunde von East an *Nicotiana*. East (1933) konnte bei verschiedenen, hinsichtlich der *S*-Allele homozygoten Genotypen von *Nicotiana sanderae* bei konstanter Temperatur nach Selbstung eine unterschiedliche Wachstumsgeschwindigkeit der Pollenschläuche beobachten; ferner berichtet er (1932) über die Wirkung von 3 Modifikationsfaktoren A_1, A_2 und A_3, die die Aktion verschiedener *S*-Allele von *Nicotiana* einschränken. Seine Befunde zeigen, daß die Wirkung der *S*-Allele und damit das Pollenschlauchwachstum durch die Art des Genotyps beeinflußt werden kann.

Die Annahme, daß die *F*-Faktoren ihre Wirkung auf die *S*- und *Z*-Allele nur ausüben können, wenn sie neben diesen in heterozygoter Form vorliegen, war notwendig, um die angenäherten 1:1 Spaltungsverhältnisse zu erklären. Sie läßt sich physiologisch als Heterozygotieeffekt deuten.

III. Pollenkeimung in vivo und in vitro

Die Inkompatibilität beruht auf einer Reaktion zwischen Pollen und Gynoeceum, die entweder bereits auf der Narbe stattfindet oder sich erst im Griffel- bzw. Fruchtknotengewebe abspielt. Übereinstimmend mit den Befunden an *Brassica*, *Capsella* und *Raphanus sativus* konnten durch die vorliegenden Untersuchungen für *R. raphanistrum* folgende 3 Tatsachen sichergestellt werden (Tabelle 6):

1. die Sterilitätsreaktion zwischen Pollen und Gynoeceum findet schon auf der Narbe statt,
2. die Pollenkeimungsrate ist reduziert,
3. die Keimgeschwindigkeit des keimenden Pollens ist verzögert.

Sears (1937) und Tatebe (1939) prüften als erste, ob die Hemmung nach Selbstbestäubung selbststeriler Cruciferen nur auf der Narbenoberfläche oder auch in dem Narbengewebe vorliegt. Sears konnte bei *Brassica oleracea* feststellen, daß die Entfernung der obersten Schicht des Narbengewebes bereits genügt, um die Hemmungsreaktion aufzuheben. Zahlenangaben, aus denen die Wirkung der Entfernung verschieden großer Narbenteile zu ersehen wäre, liegen nicht vor. Tatebe führte ähnliche Experimente an *Raphanus sativus* durch. Seine Ergebnisse sind zahlenmäßig erfaßt. Sie zeigen, daß er den höchsten Fruchtansatz mit 87% nach Entfernung der halben Narbe erzielen konnte. Dieses Resultat entspricht dem Bestäubungsergebnis, das wir an *R. raphanistrum* durch Anwendung von Methode V (Einführung des Pollens in das Narbengewebe) erreicht

haben. Der Selbstbestäubungserfolg nach dieser Methode (80%iger Fruchtansatz) ist ein Beweis dafür, daß auch bei *R. raphanistrum* das hemmende Prinzip auf die Narbenoberfläche beschränkt ist.

Die Voraussetzung für eine Klärung der stofflichen Grundlage der Selbst- und Kreuzungssterilität war die Reproduktion der nach Selbstbestäubung stattfindenden Reaktion *in vitro*.

Correns (1913) hat als erster diese Versuchsrichtung eingeschlagen. Er erhielt auf einem Gelatine-Rohrzuckermedium, dem er vor Aussaat von *Cardamine pratensis*-Pollen einen Quetschsaft aus Narbenköpfen des Pollenspenders zusetzte, eine Keimungshemmung. Die Hemmung erwies sich aber als unspezifisch. Yasuda (1928/29) berichtet in zwei Arbeiten über erfolgreiche Versuche, die Keimung von *Petunia*-Pollen in Rohrzuckerlösung durch Zusatz von Narbensekret und Griffelgewebe des Pollenspenders gegenüber dem eines Individuums von einer anderen Linie zu reduzieren. Aus diesen Versuchen schloß er auf das Vorhandensein eines Hemmstoffes in dem Griffel, der nur auf Pollen der gleichen Pflanze wirkt. Yasudas Versuche konnten jedoch von Straub (1946) nicht reproduziert werden. Straub stellte wohl eine die Pollenkeimung hemmende Wirkung nach Zusatz von Griffelgewebe zu der Zuckerlösung fest; die Wirkung von Griffelgewebe derselben Pflanze war jedoch unspezifisch. Tatebe (1947) konnte für *R. sativus* die nach Selbstbestäubung auf der Narbe stattfindende Hemmung *in vitro* reproduzieren. Als Keimmedium verwandte er Rohrzuckergelatine, der zerquetschtes Griffelgewebe zugesetzt war. Die Keimung von eigenem Pollen war auf diesem Medium vollständig gehemmt. Kontrollversuche mit Griffelgewebe von Pflanzen, die sich mit dem betreffenden Pollen befruchten ließen, wurden von ihm nicht durchgeführt. Als Kontrollmedium diente ihm Griffelgewebe aus Knospen und älteren Blüten, die gemäß den Ergebnissen seiner Pseudofertilitäts-Untersuchungen pseudofertil waren. Er erhielt auf diesem Medium eine Pollenkeimung. Wenn man berücksichtigt, daß der von Tatebe verwendete Quetschsaft nur zu einem sehr geringen Teil hemmende Substanzen enthalten konnte, da er nicht nur aus Narben, sondern aus ganzen Griffeln hergestellt worden war — im Griffel besteht nach den Versuchen Tatebes keine Hemmwirkung —, dann sind die von Tatebe erzielten Resultate nicht sehr überzeugend. Versuche von Sears (1937), die nach Selbstung auf der Narbe stattfindende Hemmreaktion *in vitro* ablaufen zu lassen, waren erfolglos.

Die in der vorliegenden Versuchsserie angewandte Methodik der „Narbengelatine" brachte insofern einen Erfolg, als sich unter Berücksichtigung des gesamten Versuchsmaterials die Pollenkeimung *in vitro*, entsprechend dem Verhalten *in vivo*, auf eigener „*Narbengelatine*" mit einem P-Wert von $< 0{,}001$ gut gesichert von der auf fremder „Narbengelatine" unterschied. Dieses Versuchsergebnis zeigt, daß sich die Reaktion zwischen Pollen und Narbe nach Selbstbestäubung *in vitro* reproduzieren läßt, wenn eine geeignete Methode angewandt wird. Offenbar kommt es darauf an, eine Zerstörung des Gewebes, welches die Hemmreaktion liefert, zu vermeiden.

IV. Physiologische Grundlage der Sterilitätsreaktion

Bei Organismen, deren Schlauchhemmung nach Selbstbestäubung im Griffel stattfindet, scheint die Hemmung durch eine Immunitätsreaktion zwischen Griffel und Pollenschlauch zustande zu kommen (Linskens 1955). Bei solchen Objekten

findet sich keine lokal begrenzte Hemmzone vor; der Griffel vermag vielmehr in jeder Höhe einen Hemmeffekt auf Selbstschläuche auszuüben (Emerson 1940, Straub 1946). Es erhebt sich die Frage, wie sich die Versuchsresultate über die Pollenkeimung von *R. raphanistrum* auf der Basis einer Immunitätsreaktion erklären lassen. Da bereits die Keimungsrate des Pollens nach Selbstbestäubung eine Reduktion erfährt, muß die Immunitätsreaktion schon kurz nach Aufbringen des Pollens auf die Narbe eintreten. Voraussetzung für das Zustandekommen einer solchen Reaktion ist das Vorhandensein eines genabhängigen Antigens und Antikörpers. Da sich bei *Brassica* und *Raphanus* die Hemmung ausschließlich auf die Narbenoberfläche beschränkt, ist eine Anwendung des Immunitätsmechanismus zur Erklärung der Sterilitätsreaktion nur möglich, wenn man annimmt, daß die Antikörper nur in der oberflächlichen Narbenschicht gebildet werden, oder nur dort in reaktionsfähiger Form vorliegen. Die Immunitätsreaktion könnte so zustande kommen, daß spezifische Stoffe sowohl von der Narbenoberfläche als auch im Pollen in Abhängigkeit von den *S*- bzw. *Z*-Allelen gebildet werden. Nach einer Bestäubung wird ein Teil der spezifischen Pollensubstanzen abgegeben. Durch ihren Kontakt mit den von der Narbenoberfläche gebildeten Stoffen kommt es zu einer Antigen-Antikörperreaktion, die sich auf die Pollenkeimung und das Schlauchwachstum hemmend auswirkt. Das Reaktionsprodukt könnte auf verschiedene Weisen wirksam werden. So könnte es, wenn es nicht selbst direkt hemmend wirkt, z. B. auf der Narbe eine p_H-Verschiebung nach der sauren Seite hin auslösen, die nach Branscheid (1930) eine Hemmung der Pollenkeimung nach sich ziehen würde.

Eine andere Möglichkeit der Erklärung der Hemmreaktion auf der Narbe ist durch die Annahme eines Hemmstoffes gegeben, der sich unter der alleinigen Einwirkung der im Gynoeceum vorliegenden hemmenden Allele von vornherein bildet, und zwar ausschließlich in der oberflächlichen Narbenschicht. Dem Hemmstoff müßte ferner eine ganz spezifische Wirkung zugesprochen werden, da er nur die Keimung und das Schlauchwachstum von Pollen hemmt, der das gleiche Allel wie die Narbe enthält. Im Gegensatz zu der Immunitätsreaktion würde es sich in diesem Fall nicht um eine Wechselwirkung zwischen Pollenkorn und Narbenoberfläche handeln; die Narbenoberfläche wäre vielmehr befähigt, einen Stoff auszubilden, der bereits ohne den Anreiz genkontrollierter Pollensubstanzen hemmenden Charakter trägt.

Eine Entscheidung darüber, ob es sich bei der Sterilitätsreaktion um einen Immunitätsmechanismus handelt, oder ob sie die Folge einer spezifischen Hemmstoffbildung ist, kann vorläufig nicht getroffen werden. Sie ist erst möglich, wenn nachgewiesen wird, daß entweder nach Selbstbestäubung eine spezifische Komponente in der Narbenoberfläche auftritt, die als Reaktionsprodukt von Antigen und Antikörper angesehen werden kann, oder wenn ein Stoff in der Oberflächenregion einer unbestäubten Narbe aufgefunden wird, der je nach der genetischen Konstitution der Narbe einen spezifischen Bau aufweist. Im ersten Fall müßte es sich um eine Immunitätsreaktion handeln, während der zweite Befund für das Vorhandensein eines spezifischen Hemmstoffes sprechen würde.

Zusammenfassung der Ergebnisse

1. Die genetische Deutung der Selbst- und Kreuzungssterilität erfolgte mit Hilfe der Annahme von 2 hemmenden Allelenserien *S* und *Z*. Der *S*- und *Z*-Serie

gehören je 4 Allele an. Der Pollen ist sporophytisch determiniert. Zwischen den Allelen jeder Serie bestehen Dominanzverhältnisse. Im allgemeinen ist die S-Serie epistatisch über die Z-Serie. Unter gewissen genetischen Konstellationen kann jedoch das Z_1-Allel epistatisch über S_1 und S_2, das Z_2-Allel epistatisch über S_2 sein.

2. Zur Deutung der erblichen Pseudofertilität wurde zusätzlich noch ein weiteres Faktorenpaar F_1F_2 angenommen. Wenn sich die F-Faktoren in heterozygoter Form neben den S- und Z-Allelen vorfinden, vermögen sie die Wirkung der hemmenden Faktoren zu modifizieren, so daß es zu einem Fruchtansatz nach Selbstbestäubung kommen kann.

3. Nach Selbstbestäubung findet die Sterilitätsreaktion zwischen Pollen und Gynoeceum bereits auf der Narbe statt; die Pollenkeimungsrate ist reduziert und die Keimgeschwindigkeit des keimenden Pollens extrem verzögert.

4. Unter Umgehung der Narbenoberfläche konnte durch 4 verschiedene Methoden der Selbstbestäubung ein Fruchtansatz bei selbststerilen Pflanzen erzielt werden. Seine Höhe ist von der Bestäubungsmethode abhängig.

5. Aus der Tatsache, daß bei Einführung des Pollens in das Narbengewebe ein Fruchtansatz von 80% erfolgte, wurde geschlossen, daß die Hemmreaktion auf die Oberfläche der Narbe beschränkt ist.

6. Selbstbestäubungen von Knospen zeigen das Vorhandensein von Knospen-Pseudofertilität; dagegen konnte keine Pseudofertilität alternder Blüten oder älterer Pflanzen („end-season-fertility") beobachtet werden.

7. Bei dem Versuch, die auf der Narbe nach Selbstbestäubung stattfindende Hemmreaktion *in vitro* mit Hilfe von „Narbengelatine" zu reproduzieren, zeigte sich eine gesicherte Differenz zwischen der Pollenkeimung auf eigener und der Keimung auf fremder „Narbengelatine". Auf Grund dieses Ergebnisses wurde angenommen, daß wenigstens ein Teil der nach Selbstung in der Narbenoberfläche wirksamen Substanzen in die Gelatine übergetreten sein muß.

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SELECTION FOR ENVIRONMENTAL VARIABILITY OF BODY SIZE IN MICE

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With 2 figures in the text

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Introduction

Selection in both laboratory and domestic animals is generally directed towards one extreme: parents for the next generation are chosen because they have the greatest deviation in the desired direction from the population mean. The primary consequence will be a change in the frequency of genes affecting the mean expression of the selected character. The selection may, however, change the frequencies of genes of another type—those which control the susceptibility of the individual to environmental factors influencing the character. Such genes would tend to be more frequent in the more extreme deviants which would be selected as parents. Changes in the frequencies of these genes would not affect the mean of the selected character, but would be manifest in an increase in environmental variation—a possibility that has been foreseen and discussed by Haldane (1931). Increases of phenotypic variance (or coefficient of variation) have often been found in selection experiments. MacArthur's (1949) experiment with mice may be cited as an example. But in ordinary selection experiments it is not easy to decide whether a change of variance is a consequence of the change of mean or indicates an increased sensitivity to environment. It therefore seemed desirable to investigate the effect of selection of extremes without at the same time changing the mean level of the character. This was done by selecting opposite extremes and mating them together. If genes that affect sensitivity to environment without affecting the mean are important we would expect to obtain an increase of phenotypic variance by applying this type of selection.

The experiment was done with mice and the character selected was body-weight at 6 weeks of age. Selection of opposite extremes was carried through 13 generations and the variance of 6-week weight in the "extreme" line was compared with that in a "central" line in which individuals with the least deviation from the mean were used as parents. About two thirds of the phenotypic variance of the character studied was found to be environmental in origin, so if genetic differences in susceptibility to environmental influences are important they ought to be brought to light in the experiment.

Since the experiment was begun, interest in genetic differences in sensitivity has developed further, particularly in connection with inbreeding (see for example Reeve and Robertson 1953 and Grüneberg 1954). The present experiment.

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however, suggests that genes affecting only sensitivity do not add an important complication to the interpretation of selection experiments, at least in their early stages.

Procedure

Stock. A heterogeneous stock of mixed origin was used. It was made by crossing four unrelated outbred stocks. One of these was the cross-bred strain derived from Goodale's and MacArthur's large strains (see Falconer and King 1953); another was a stock selected for high lactation (Bateman, unpublished); and the other two were stocks of mutants. The foundation population, designated generation 0, was the double cross from the two first crosses.

Mating System. Two lines were separated from the foundation population, called the "extreme" and "central" lines. Each line was maintained by 8 pair-matings in every generation. Mice chosen to be parents of the next generation were taken equally from all families; so that, when all 8 matings bred, each contributed two offspring to be used as parents. Matings were made by least relationship based on tables of covariances. In this way inbreeding was minimized. The calculated coefficient of inbreeding increased from zero in generations 0 and 1 to 22% in generation 13. Two litters were raised from each mating, but in order to shorten the generation interval, selections were made only from the first litters. Litter-size was standardized at birth as nearly as possible to 8 mice, with 4 of each sex. Litters were weaned and the sexes separated at 3 weeks of age. The mice in any generation were mated all at the same time, as soon as the youngest had reached 6 weeks.

Selection. Selection was based on body-weight at 6 weeks of age, and was made entirely within litters. That is to say, two mice were selected from each litter, one of each sex, and the criterion of selection was the deviation from the litter-mean, males and females being treated separately. In the extreme line this amounted to choosing the largest or the smallest male and the largest or the smallest female, the choice being determined by the deviation. In the central line, mice with the smallest deviations were chosen. When one or more matings failed to breed, extra mice were taken from the most suitable of the existing litters. The selected mice in the extreme line were mated disassortatively, members of a pair always having deviations of opposite sign. Large males were always mated to small females and small males to large females. The need to have as many positive as negative deviants and as many males as females sometimes reduced the freedom of choice in making the selections and prevented the use of the best animals in some litters. In the central line disassortative mating was applied only when animals with rather large deviations had to be used.

Results

The essential data are set out in Table 1, where the following observations are listed for each generation:

1. The average deviation of parents from their litter-means, disregarding sign. This is a measure of the intensity of selection applied to sensitivity to environmental influences. The mean over all generations was 2·08 gm. in the extreme line and 0·50 gm. in the central.

2. The mean deviation of parents taking sign into account. This is the net selection differential which would influence the mean body-weight of the succeeding generation. The mean over all generations was + 0·085 gm. in the extreme line and + 0·045 gm. in the central line.

3. The mean 6-week weight of females and of males in each line.

4. The square root of the within-litter variance in weight, calculated from an analysis of variance of each generation.

Means. The mean weights of females and of males in successive generations are shown in Fig. 1. The mean weights remained substantially equal in the two lines until generation 7; but after that both females and males of the extreme line became heavier than those of the central line. The difference increased until finally it amounted to 2·4 gm. in the females and 3·3 gm. in males. Thus the aim of maintaining a constant and equal mean weight was not achieved, and the reason for this is not clear. It is true that the net selection on weight was slightly greater in the extreme line than in the central (see Table 1), but the divergence in weight is far too great to be

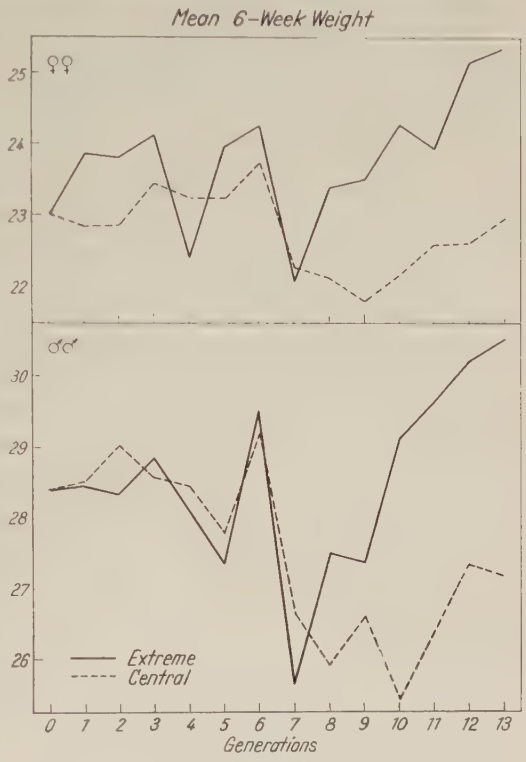

Fig. 1. Mean 6-week weights (gms.) in successive generations

Table 1

Generation	Mean parental deviation		Net selection differential		Mean Weights				Standard deviation (within litters)			
					E		C		E		C	
	E	C	E	C	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
0	—	—	—	—	—	—	23·0	28·4	—	—	1·34	2·93
1	2·66	0·34	+ 0·38	+ 0·01	23·9	28·5	22·8	28·5	1·13	2·38	1·31	2·22
2	1·71	0·41	− 0·01	+ 0·11	23·8	28·4	22·9	29·0	1·36	2·10	1·48	2·26
3	1·68	0·51	+ 0·38	+ 0·19	24·1	28·8	23·4	28·6	1·72	1·87	1·49	2·39
4	2·01	0·62	− 0·32	+ 0·12	22·4	28·1	23·3	28·5	1·76	2·95	1·50	2·33
5	2·78	0·51	+ 0·34	+ 0·08	24·0	27·4	23·2	27·8	1·74	2·52	2·02	2·20
6	2·13	0·71	− 0·14	+ 0·05	24·3	29·5	23·8	29·2	1·84	2·96	1·43	1·86
7	2·64	0·86	− 0·20	− 0·08	22·1	25·7	22·3	26·6	1·28	1·99	1·76	1·65
8	1·95	0·30	− 0·15	− 0·12	23·4	27·5	22·1	25·9	1·35	2·02	1·42	2·42
9	2·11	0·43	− 0·09	+ 0·07	23·5	27·4	21·8	26·6	1·72	3·17	1·38	0·98
10	1·39	0·52	+ 0·23	+ 0·05	24·3	29·1	22·2	25·4	1·62	2·59	1·47	1·66
11	2·10	0·33	− 0·03	− 0·01	24·0	29·7	22·6	26·4	1·45	2·61	1·46	1·59
12	2·25	0·51	+ 0·74	+ 0·16	25·1	30·2	22·6	27·4	1·66	1·98	1·29	1·79
13	1·69	0·43	− 0·03	− 0·04	25·4	30·5	23·0	27·2	1·93	2·70	1·45	2·01
Mean	2·08	0·50	+ 0·085	+ 0·045								

attributed to differential selection, which over all generations amounted to only 0·5 gm. Again, the difference seems to be too great to be attributable to the effects of random drift. The expected effect of drift can be roughly estimated from the calculated coefficient of inbreeding (F = 22% at generation 13) and from an estimate of the total additive genetic variance of weight ($\sigma_G^2 = 2{\cdot}28$ gm.^{2}) derived from another experiment with the same stock. The betweenline variance expected from the effects of drift ($2F\,\sigma_G^2$) is then 1·00 gm.^{2}, and the standard deviation of the expected difference between two lines is 1·41 gm., which is less than half the observed difference.

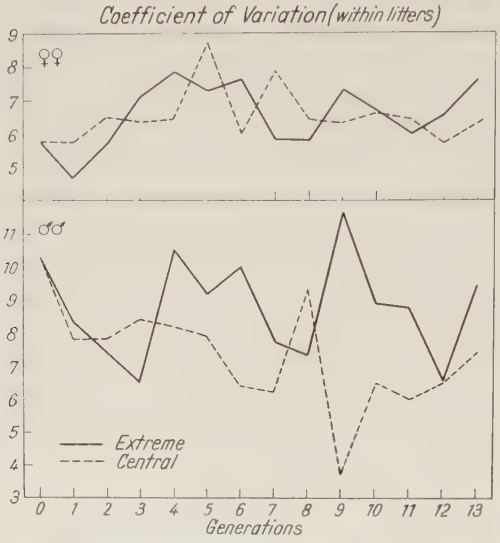

Fig. 2. Coefficient of variation within litters of 6-week weight in successive generations

Variances. Variances of weight are known to be correlated with mean weights in mice (MacArthur 1949, Falconer 1953). Therefore in order to minimize any effects arising from differences in mean weight, the variances were converted to coefficients of variation $\left(= \frac{\text{standard deviation}}{\text{mean}} \times 100\right)$. The variance within litters was thought to be the best criterion for comparison. Fig. 2 shows the coefficient of variation, within litters, of females and of males in the two lines. No differences of variation between the extreme and central lines is apparent in females, but the males are more variable in the extreme line, at least from generation 4 onwards. A simple average of the values for each generation over the whole experiment gives a mean coefficient of variation of 8·64% in the extreme line and 7·09% in the central line. To test the significance of this difference we compare it with its standard error, using the between generation variation as error. The difference is 2·7 times its standard error and is therefore significant.

Table 2. *Average coefficient of variation in first and second halves of the experiment*

	Males		Females	
	generations 1–7	generations 8–13	generations 1–7	generations 8–13
	%	%	%	%
Extreme . . .	8·7	8·6	6·7	6·5
Central . . .	7·8	6·6	6·6	6·5

Looking further into the source of the difference of variation in males we find it to be due to a decrease of variation in the central line, rather than to an increase in the extreme line. In Table 2 the experiment is divided into two parts, the first 7 and the last 7 generations, and the mean coefficient of variation in the two parts is given. There is no tendency for the variance to increase in the ex-

treme line in either sex; in fact it decreases slightly in both sexes. But in the central line there is a decline of the coefficient of variation in males, though not in females.

The interpretation of these results is far from clear, and before attempting to draw a conclusion from the experiment, we shall examine more closely the expected consequences of the mating system.

Discussion

This investigation was designed to attack the problem of selection for genes controlling sensitivity to environmental influences. It was realised, however, that the mating systems used may also affect the frequencies of other types of genes and gene combinations. MATHER (1943) has argued that the higher fitness of metric intermediates will lead to the building-up of repulsion linkages between genes affecting the character in the same direction. In our case, this would mean a decrease of free genetic variance in the central line and an increase in the extreme line by the building-up of repulsion and coupling associations respectively. But with the large number of chromosomes of mice, it would not be expected that this effect would be important in our experiment.

The type of assortative mating that we have used can also lead to changes in the frequencies of genes that affect the character directly. MOREE (1953) was the first to point out that the mating of animals of opposite extremes will, in the case of a major recessive gene, lead to a continual segregation at that locus. A more thorough investigation of the effects of such mating systems has recently been made (A. ROBERTSON, in press), from which it appears that continual selection of individuals with one fixed value of a character leads to the eventual fixation of genes affecting that character. Similarly, the "extreme" type of mating system leads to the maintenance of genetic segregation with the frequencies of additive genes approaching a stable value of 0·50 and recessive genes a value of 0·71. The gene frequencies for maximum expression of genetic variance for such genes are 0·50 and 0·75 respectively so that "extreme" matings will lead to an increase of genetic variance. A simple expression can be obtained for the change in gene frequency of an additive gene in continued extreme mating. If q is the gene frequency, then the change of gene frequency on one-way selection is equal to $\frac{i}{2}\frac{\delta}{\sigma} p q$ where i is the selection intensity, δ the effect of the gene (expressed as the difference between the two homozygotes), σ is the standard deviation of the remaining variation, and $p = 1 - q$. If we now mate together the two extremes, picking the same proportion at the extremes as in one way selection, the change is approximately $\frac{i^2 \delta^2 p q (p-q)}{8 \sigma^2}$. Rather more simply, we can say if the gene frequency is close to one then the effective selective advantage of a gene in extreme selection is approximately $\frac{s^2}{2}$, where s is the advantage under one-way selection with the same extreme proportion selected. We can conclude that the effect should be comparatively unimportant unless genes with large effects are present. A gene with frequency 0·2 and homozygote range of one standard deviation of the population will change in frequency in the extreme

line by 0·1 in 10 generations. The change in the variance due to this gene will only increase the total variance by 3%. There will be a corresponding decline in the central line by 4%.

In addition to the effect on sensitivity to environment, there are therefore two possible consequences of these mating systems through their effects on genes contributing additive variance of the character selected. Both consequences lead to greater variation in the extreme than in the central line, but the expected difference from both causes is small. Our results are by no means conclusive but, as this is the first experiment of its kind, it seemed worthwhile to report them in detail. They show no tendency for the variation to change in either line. except that the males of the central line show a decrease in coefficient of variation. The interpretation of the results is complicated by the discrepancy in the behaviour of the two sexes, and by the divergence of mean weight that started at generation 8. In spite of these complications it seems safe to conclude that in the course of selection in the extreme line we have not materially changed the sensitivity to environmental influences. The problem we had in mind at the outset was whether unidirectional selection can act on genes affecting sensitivity to environment, and thus lead to an increase of environmental variance. Our results suggest that this is not likely to be an important effect of unidirectional selection. This conclusion is in accord with evidence published since the experiment was started, which shows that the increase of variation often found in selected lines is in many cases due to unfixable genetic variation that has perhaps been magnified in effect by the selection (see, for example, Robertson and Reeve 1952, Clayton, Morris and Robertson 1954).

Summary

1. An experiment with mice is described which was intended to show whether the degree of sensitivity to environmental influences can be changed by selection of extreme phenotypic deviants for a metric character.

2. Body weight was taken as a metric character and opposite extremes (large × small) were selected as parents in the "extreme" line. Individuals closest to the mean were selected as parents in the "central" line. The coefficient of variation of body weight was followed through 13 generations of this selection.

3. No change of variability was detected in the extreme line. The males, but not the females, of the central line showed a small decline of variability.

4. The two lines diverged in body weight in both sexes in the second half of the experiment, the extreme line being the heavier. The cause of this difference is not known.

5. The conclusion to which the results point is that selection of extreme phenotypic deviants did not materially change the sensitivity to environmental influences. An increase in environmental variance from this cause is therefore unlikely to be an important consequence of unidirectional selection.

Acknowledgements. The technical work of this experiment was done by Mr. J. H. Isaacson, and the statistical computation by Mr. W. S. Russell, to both of whom we are greatly indebted.

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THE ANALYSIS OF INDUCED CHROMOSOME ABERRATIONS BY MAIZE ENDOSPERM PHENOTYPES

By

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With 1 figure in the text

(Eingegangen am 3. Oktober 1955)

Introduction

The several methods that have been used for studying induced chromosome breaks can be separated into rather distinct classes, each supplying different kinds of information. For example, the study of salivary gland chromosomes of F_1 larvae in *Drosophila* gives in minute detail the positions of breaks, but entirely disregards aberrations incapable of going through mitosis. Purely genetic studies in *Drosophila* have this last property also and, in fact, the screening out of various chromosome recombinations, in unknown frequency, into a lost class of dominant lethals produces a major difficulty of interpretation (Fano 1947). In contrast, *Tradescantia* chromosomes are normally studied in the metaphase immediately following irradiation so that all aberrations including thoses unable to go through anaphase are observable. On the other hand, the observations themselves are limited as to detail, often subjective, and difficult to handle in a manner that satisfies statistical sampling requirements.

Considered from this abstract point of view, the advantages in experimentation that might be gained from a new method, having different properties, are obvious. The purpose of this paper is to consider the study of maize endosperm mosaics as such a method.

Essentially, pollen carrying linked endosperm markers is treated, and used to pollinate a plant homozygous for corresponding recessives. An important property of chromosomes in maize endosperm is that freshly broken ends generate Breakage-Fusion-Bridge cycles which are detectable phenotypically. Correspondingly, one of the chief interests of the method, in which it differs from others, is that free chromosome ends produced by mutagenic agents, can be identified. The endosperm phenotypes are all recognizable with a minimum of ambiguity, and the observational data themselves can be described as very objective. The interesting properties of the method are only realized when several markers are used simultaneously in the same chromosome arm: one unlinked marker yields but limited information. The choice of linked endosperm markers at present available is very limited, and when the further necessary restriction is imposed that the phenotype produced by the loss of combinations of markers be unambigously identifiable, it is found that only one chromosome arm approaches the requirements: the short arm of chromosome 9. The method is thus in practice restricted to this relatively small length of chromatin. In endosperm, this is genetically covered by two normal chromosomes carrying the recessive markers.

and under these circumstances, even the complete loss of the arm causes no appreciable viablity handicap in aleurone development.

The endosperm analysis method is quite economical in terms of the amount of time and labor expended for each aberration. Nevertheless, it does not appear to be very suitable for large scale purely quantitative work involving, for example, extensive ranges of doses. It should therefore be regarded primarily as a cytogenetical, rather than a radiological tool. The numerical precision attainable in the method is limited; also, a number of classes of aberrations, distinguishable in other experimental arrangements, have to be bracketed together.

Endosperm analysis was used by the writer for comparing the action of X-rays and ultra violet, and the data of these experiments will be discussed here. The specific task of comparing the two radiations will be dealt with separately. It so happens that the data obtained from the two kinds of treatment are very much alike, though not identical. The present purpose is to discuss the method as such; the differences between the treatments will not be dealt with. It will be seen that in many instances, statistical tests permit the pooling of data from the two sources.

Literature

Spontaneous mosaics and whole kernel losses for endosperm character have been object of interest since the first years of maize genetics, and various conjectures about their origin are to be found in the early literature. Two papers of this period are by Emerson (1915 and 1921); in the last one, Emerson made the correct conjecture, that areas from which a dominant character is lost result most often from aberrant chromosome behavior. Even before, East and Hayes (1911) had pointed out the analogy between endosperm mosaics and gynandromorphs in insects. This early work is reviewed by Jones (1937). Some of the earliest genetic effects of radiation to be described, were observed by Stadler (1928) in maize endosperm. From the fact that the linked dominant markers *C* and *Wx* were rather often lost simultaneously, and that the total rate of induced losses was very high, Stadler also deduced that these effects are largely or almost entirely due to loss of chromosome segments, a conclusion which subsequent work entirely confirmed. In a later paper, Stadler (1930) deals with a special phenomenon in endosperm mosaics, both spontaneous and induced, consisting essentially of small island of tissue carrying dominant markers in a larger field form which these markers were lost. These he called "Recoveries". It is not easy to decide from the descriptions given exactly what kernels were being examined in all these cases. The statement, "The frequency of typical endosperm chimaeras (including all those in which the deficient sector occupies approximately one half of the endosperm or less) is not materially higher in seeds produced by the use of X-rayed pollen than in untreated seeds", is difficult to reconcile with the author's own later experience (Stadler and Sprague 1936, Stadler and Uber 1942) and indeed with any one else's experience with this material. In the light of more recent information, it would seem that these "recoveries" probably belonged to several distinct classes, and that their origin is attributable to a number of diverse mechanisms. Stadler and Sprague (1936) were chiefly concerned with comparing ultra violet with X-ray action, and do not discuss the mechanism of mosaic production at any length. For the

most part unlinked markers were used, and losses classified according to whether the loss area, as a proportion of the whole kernel, was about $^{15}/_{16}$, $^{7}/_{8}$, $^{3}/_{4}$, $^{1}/_{2}$, $^{1}/_{4}$, $^{1}/_{8}$, $^{1}/_{16}$ and "small" and it was found that the median for all mosaics is about $^{1}/_{2}$. When *C* and *Wx* were used together on the same chromosome, it was noted that they were often associated in loss, and from this it was again concluded that usually these phenomena are chromosomal rather than genic. In the later work of Stadler and Uber (1942) losses were more simply classified into "entire" and "fractional"; this work had as its principal aim the establishment of an ultra violet action spectrum, and used only the unlinked markers *A*, *Pr* and *Su*. Thus, there were few opportunities to detect chromosomal aberrations, but the authors again express their conviction that few, if any, of the observed losses are ascribable to mutation, because of observations on the mosaic pattern given by the loss of markers simultaneously. In all of Stadler's work, the only linked endosperm markers used were *C* and *Wx*. This is not an ideal combination, for while loss of *Wx* can easily be looked for in an area of *C* loss, it is inconvenient, on the necessary scale, to search for *Wx* losses under the highly colored aleurone given when *C* is present. Partly for this reason, the very characteristic mosaic pattern given by Breakage-Fusion-Bridge cycles, which constitute a large proportion of all radiation induced mosaic kernels, and must have been present in large numbers, was not recognized in any of Stadler's work. Hence it is usually difficult to decide exactly what phenotypes were classified by him under the heading of mosaics. If different dosages of some dominant marker are recognizable phenotypically, and if the effect does not diffuse too much from cell to cell, such a marker will exhibit twin spotting when involved in a B.-F.-B. cycle. Used in the right background, *C* is such a marker and beautiful twin spots can be observed in large numbers when *C* is used in radiation experiments. Nevertheless, twin spots are not mentioned in any of Stadler's papers referred to here. Jones (1936 and 1938) described twin spots for *C*, and attributed them "not . . . to mutation or loss, but to an unequal mitosis"; the term "unequal mitosis" would apply quite well to the B.-F.-B. cycle, which is now known to be the usual underlying mechanism. But this was not known to Jones, who looked for an explanation in some other chromosomal phenomenon related to the somatic crossing over of *Drosophila*, despite the fact, that special experiments to detect exchange between homologous chromosomes, using for instance *C wx* male on *c Wx* female, yielded completely negative results. Many illustrations of twin spots are given in Jones (1937). Jones (1941) investigated endosperm marker losses caused by X-raying pollen. He wrote: "These changes, both natural and induced, are for the most part the result of the removal of whole parts of chromosomes, as shown by the linked genes that are taken out in the same order and in almost the same frequency as they are rearranged in meiosis." While in the light of later work, this is an oversimplification, it is the first recognition of the fact that the rate of induced loss is related to position on the chromosome. The B.-F.-B. cycle mechanism was still not considered, although it had by then been described, but the essential fact that in a mitotic incident, one daughter cell gained what the other lost, was recognized. McClintock's (1938a) discovery of the B.-F.-B. cycle was an essential step in the understanding of mosaic patterns. A more complete examination of this mechanism is given in McClintock (1941). In

McClintock (1942), the distinction is made between *chromatid* and *chromosome* B.-F.-B. cycles, the latter being more particularly characteristic of the zygote, rather than the endosperm. A direct cytological demonstration of chromatid bridges in endosperm mitoses, produced by B.-F.-B. cycles, was given by Clark and Copeland (1940). In McClintock's early work, B.-F.-B. cycles were produced by free chromosome ends, generated by the breaking of an anaphase bridge following crossing over in an apocentric (= paracentric) inversion. Later McClintock (1950, 1951) was able to produce free chromosome ends by the *Ds-Ac* combination. With this material, B.-F.-B. cycles in the short arm of chromosome 9 can be produced in every kernel of an ear. The study of this abundant material, with various marker combinations, has made it possible to identify the phenotypes of B.-F.-B. cycles with great reliability. An important contribution was McClintock's introduction of the marker combination *I Sh Bz Wx*, all in the short arm of chromosome 9, which are of such a nature that many combinations of losses among them are separately recognizable. In these last two papers, the endosperm phenotypes produced by number of genetically controlled mutable loci are described, and consideration is given to the means of distinguishing these from the mosaics given by B.-F.-B. cycles.

We have insisted on the agreement among all the authors so far quoted, that radiation induced endosperm marker losses are of chromosomal rather than genic nature, in the great majority of cases. Rather remarkable exceptions to this are the work of Singleton (1951, 1954) and Dollinger (1954), who refer to such marker losses as mutations. While the word mutation is used by them to cover any detectable alteration, as it is by the Swedish workers, their discussion makes it clear that the difference is more than a semantic one, and they appear to be of the opinion that the effects are analogous to the point mutations occurring in maize spontaneously. Two short notes on some of the material presented in this paper have been published (Fabergé 1951, 1954).

Methods

All pollinations were made in a greenhouse, with extreme precautions against contamination. Control pollinations with untreated pollen were invariably made from each tassel used, and on each day on which treatments were conducted. These controls demonstrated that the precautions were effective; they will not be mentioned further.

Irradiation. X-rays. Parts of tassels with ripe anthers were treated in glassine bags, on a paraffine block for back scatter, with 1500 R of X-rays of medium hardness (100 kV peak, two-wave rectifier, tungsten target).

Ultra violet. The contents of about three anthers were used to pollinate each ear. The pollen was spread in a single layer on a quartz slide, covered with another slide, and exposed to radiation from two mercury discharge tubes, one above, one below. The total dose incident at the surface, from both sides together, was 546,000 ergs cm^{-2}. This figure is total radiation measured with a thermopile, and includes not only λ 2537 Å, which represents most of the active radiation from the WL782 H-10 tubes, but also small quantities of other U.V. wavelengths, and visible radiation. For the present purpose, more discussion of radiation methods seems irrelevant; fuller details will be given in a later paper.

Genetic material. The use of the markers *I*, *Sh*, *Bz*, *Wx*, in chromosome 9, and the reading of mosaic patterns, was not originated by the writer, but is entirely due to McClintock, who (1951) described the phenotypes and gave photographs of kernels with various *Ds* events involving these markers. Since the present work depends on these methods, a brief description of the essential facts seems justifiable, even though they are already known to a few

workers in the field. Rhoades (1952) has described the interactions and position of the marker *bz*.

Pollen from plants homozygous for *I Sh Bz Wx* was irradiated, while the female parent was homozygous for *C sh bz wx*. These stocks were homozygous for background genes needed to give strong aleurone color.

Fig. 1 gives the cytological and genetical location of the markers. The cytological map is made from data by Rhoades and Patterson (unpublished) and the writer is much indebted to Dr. Patterson for supplying the necessary information. It should be born in mind that cytological localisation of markers in maize is rarely very precise, and absolute pachytene lengths cannot be measured with any great accuracy. *Wx* is certainly in the euchromatin not far from the thick proximal heterochromatic region. The genetic map shows that crossing over is quite drastically reduced in the region close to the centromere. The best available estimate for the genetic distance from *Wx* to the centromere (Rhoades, unpublished) is about 10–12 units. Other genetic map distances are from Rhoades (1950).

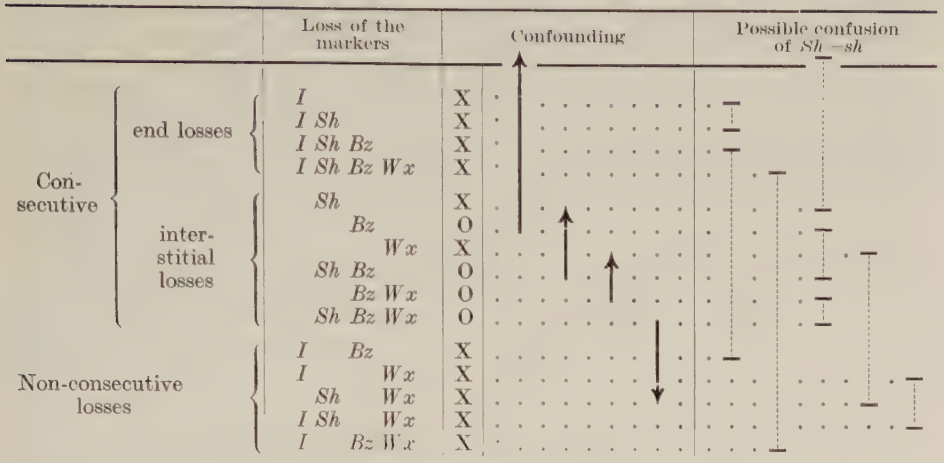
Endosperm phenotypes. McClintock (1951) has described the phenotypes produced by combinations of the markers *I Sh Bz Wx*, and given photographs of B.-F.-B. cycles involving them. In Table 1 is summarized information about the recognition of the phenotypes resulting from loss or inactivation of any combination of these four markers. The word "loss" is used here and elsewhere in this paper, simply to designate the disappearance of the phenotypic effect of the dominant allele, without any implication as to possible mechanisms. The underlying cause of a loss so defined, may be a chromosome deficiency, or a point mutation or some other genetic or cytological mechanism.

Essentially *I* and *Bz* are color genes having a rather unusual interaction, *sh* gives a characteristic pitting of the kernel, while *wx* changes the nature of the starch. *sh* and *wx* are independent of one another and of the color markers in their expression. The phenotype (*C* present) of both dominants *I Bz* together gives a light straw colored kernel. Loss of *I* alone gives deep blue aleurone if *C* is present on another chromosome, as is the case here. Loss of both *I* and *Bz* exposes *C* and *bz*, which produces a buff color, distinctly darker than the straw color of the unaffected kernel. The outermost cell layer of aleurone having this *C bz* buff color has a small amount of anthocyanin which can be demonstrated by the application of an acid (e.g. Normal HCl) to the cut surface, a test which was used at times. When straw colored tissue of *I Bz* constitution adjoins buff *C bz* tissue, a strong line of color appears between them, since *I* and *bz* apparently block the path of anthocyanin synthesis at two different places in such a manner that substances can interact after diffusion across the boundary between the two tissues (Rhoades 1952).

A cross in the table indicates that the corresponding genotype can be identified without any ambiguity. O indicates that the genotype is confounded with the one shown by the full arrow. This last is the one always used in the summary of data table (Table 2); that is, in each case, the minimum loss is assumed, purely as a convention. It will be seen that the basic reason for the confounding is that the loss of *Bz without* the loss of *I* is not identifiable on this background, except under special circumstances. The special condition is, that while in a mosaic of *I Bz* tissue with *C bz* tissue, a line of anthocyanin appears along the border, no such color line appears between *I bz* tissue and *C bz* tissue. In each case. *C* is present in double dose, from the female side. Thus, in some of the more complex aberrations, the loss of *Bz* alone is detectable, by an interruption of the color line. This is particularly well demonstrated in ring chromosomes.

Except for the well defined confounding indicated, the classification of *I*, *Bz* may be said to be free of any ambiguity, and to involve no subjective judgement. The same is true of *Wx*; it can in the first instance be detected by a difference in light diffusion, with side illumination and a knife blade to cast a shadow. Iodine on a cut surface is used for a critical test. All the apparently normal kernels showing no loss for *I*, *Sh* and *Bz*, were stained in iodine in bulk; they were first soaked for 24 hours in 2% aquaeous KI, then this solution was replaced by a KI + I_2 solution containing about 0·2% iodine. By this means, losses and mosaics for *Wx* alone were reliably detected. The difficulty of applying such a test to the *C Wx* material used by Stadler, led to disregard of the losses of *Wx* alone. The degree of expression of *Sh-sh* depends somewhat on background; while the stocks used were good in this respect, very occasional confusion is possible. When involved in a cycle together with

Table 1. *Simple losses*



It is assumed that all genes necessary for aleurone color are present, and that *C* is present in two doses on the chromosome coming from the female side.

other markers, the depressions caused by *sh* are very characteristic. On the other hand, B.-F.-B. cycles for *Sh-sh* alone, without other markers to serve as indicators, would not be reliably detectable. Whole kernel losses of *Sh* are probably quite reliable. In the last column of Table 1, such possible confusions are indicated; in practice, they are believed to be fairly rare.

Reading of mosaic patterns. It is rather remarkable that a good illustration of a kernel with a B.-F.-B. cycle for markers in one chromosome arm will be found in Fig. 5 of the *Theory of Games and Economic Behavior* by VON NEUMANN and MORGENSTERN; a diagram of the underlying cell lineage is also given in Fig. 8 of the same work. The fact that all kernel mosaic phenotypes can be exactly described in terms of the Theory of Sets is mathematically quite trivial, and probably has no fruitful application. It does, however, illustrate the essential points in the reading of mosaic patterns. If the surface of the aleurone be regarded as a set whose elements are cells of various phenotypes, then loss areas of several kinds can be regarded as subsets. In particular, in the case of a B.-F.-B. cycle initiated distally to several markers in the same arm, areas of loss of the marker nearest the centromere are a *partition* of the areas of loss of the next marker, a *subpartition* of the loss of the third, a *subsubpartition* of the loss of the fourth . . . going outwards from the centromere. In our case, the system of sets of loss of *Wx* are a partition of the system of sets of loss *Bz*, which in turn are a partition of the sets of loss *I*. Using the notation proposed at the end of this section, we can write, for example:

$$\mathrm{Cy}(\mathrm{E}\,I) = \mathrm{L}(I) \supseteq \mathrm{L}(I\,Sh) \supseteq \mathrm{L}(I\,Sh\,Bz) \supseteq \mathrm{L}(I\,Sh\,Bz\,Wx).$$

Partition is here used in the sense of VON NEUMANN, there being no necessary requirement that the sum of the elements of a partition be the entire set. This requirement is sometimes fulfilled, but more typically is not. Ordinarily in the case of a B.-F.-B. cycle, a partition of each category is a system of pairwise disjunct sets, but at times consists of one set only. A departure from the partition-subpartition relationship indicates something other than a simple B.-F.-B. cycle. For example: if loss of *I* is found to be a partition of loss of *Wx*, then the chromosome segment in which the B.-F.-B. cycle occurs is inverted, *I* nearer the centromere and a free and distal to *Wx*. If two markers are lost in a mosaic pattern without either being a partition of the other, then there are two independent B.-F.-B. cycles. Many other relations could be listed. It is not proposed to expand such a treatment or to make use of the Theory of Sets and Boolean algebra, since it seems certain that common sense is a perfectly adequate guide in practice. These remarks do serve to demonstrate that the fundamental relationship used in interpreting endosperm mosaics is that of *withinness*, which is a simple

and direct consequence of cell lineage: this is precisely the point illustrated by von Neumann's figures referred to above. The development of maize endosperm is normally very irregular, so that relatively little can be deduced from the *geometry* of the pattern. The fundamental facts are read in effect by making abstraction of geometrical relationships, and considering only the sets. Geometry provides some additional information of another sort; for instance, it may indicate whether an event happened early or late in development. McClintock (1950, 1951) used this in the analysis of *Ac* action; in this case, the differences are extreme and unambiguous. In the same way, the geometry of a mosaic may indicate whether the initiating break of a B.-F.-B. cycle was, for instance, close to the end of the chromosome or close to the marker *I*. This kind of information was generally considered too vague to use in the present work. While the development of endosperm is very irregular, it seems to be such that a particular marker loss event does not as a rule give rise to more than one corresponding area on the aleurone, to *pairwise disjunct* sets in the language of Set Theory. A system of pairwise disjunct sets generally implies several loss events. At least, it can be said that if several separate areas can result from one loss event by very irregular endosperm growth, this is rare. In contrast to the irregular geometrical relations, the partition-subpartition relation is fundamental and clearcut, and all critical deductions are based on this.

In practice, the writer has found it a most necessary preliminary to examine kernels with B.-F.-B. cycles generated by the *Ds-Ac* system, with various marker combinations and *Ds* in different position. He is greatly indebted to Dr. McClintock for making such material available to him.

Tests as to the inertness of Ds. The male parent used in these experiments carried, in addition to the markers mentioned, *Ds* in standard position, between *Wx* and the centromere. *Ac* was not present in either male or female parent, and in the absence of *Ac*, *Ds* remains totally inactive. Nevertheless, some additional checks were made.

In McClintock's (1951) experiments, "new" *Ac*'s were generated in special circumstances, and McClintock expresses the view that they may be associated with heterochromatin. Since, in irradiating pollen with the doses used here, numerous chromosome breaks are produced, presumably more or less at random in the chromosome complement, and also many B.-F.-B. cycles, it might be imagined that "new" *Ac*'s could sometimes be generated by some undefined mechanism following such breaks. It was important to know whether such phenomena happened sufficiently often to affect the experiments, and in particular to activate the *Ds* present; two kinds of tests were made.

Stocks carrying *I Sh Bz Wx*, but with *Ds* in other than standard position were irradiated and used as male parents for small batches of material. Two such stocks were used: *I Sh Bz Ds Wx* and *I Ds Sh Bz Wx*. The classes resulting from an activation of *Ds* in standard position in the normal material is the loss of all four markers; in the two special stocks, the corresponding classes are respectively: loss of *I Sh Bz*, B.-F.-B. cycle for *Wx*, and loss of *I*, B.-F.-B. cycle for *Sh Bz Wx*. No obvious relative excess of these classes was found. These tests are satisfactory as direct demonstrations that an activated *Ds* plays no large scale part in radiation effects, that would be quantitatively important in the present experiments. They are not, however, sensitive detectors of *Ac* production, if this should be occuring on a smaller scale. To test this, material of bz^{m-1} was used, at Dr. McClintock's suggestion; bz^{m-1} is an *Ac* activated mutable locus (McClintock 1951). The mutation $bz^{m-1} \rightarrow Bz$, in the presence of *C* and other background genes, gives a deep blue color which would not be missed even if it occured with a low frequency. The test was made in three ways: 1. $bz \times bz^{m-1}$, pollen irradiated; 2. $bz \times bz^{m-1}$ ear irradiated immediately before pollination; 3. $bz^{m-1} \times bz$, pollen irradiated. Preliminary tests were made using heterozygous bz^{m-1}/bz plants, in which half the kernels would be capable of detecting an effect. Later, larger scale tests of type 1 and 3 were made when homozygous bz^{m-1} material became available, in all cases, both female and male parents were homozygous for *C*. Data are summarized in Table 2. The activity of bz^{m-1} was verified by crossing to *Ac* carrying plants. In all of the tests where bz^{m-1} was placed in the presence of radiation broken chromosomes, no spot suggestive of $bz^{m-1} \rightarrow Bz$ was seen. It seems safe to conclude from these completely negative results that if new *Ac*'s can be produced by events following breaks induced at random among the chromosomes. such events must be fairly rare, and too infrequent to affect any of the data presented here. This is no way contradicts McClintock's results mentioned earlier.

Notation. For purposes of discussion, it will be convenient to use some abbreviations:

L: loss, that is, disappearance of the *effect* of the dominant marker, whatever may be the underlying cytological or genetical mechanism. Thus, L(*Wx*) designates a kernel or area still showing the effects of *I Sh Bz*, but staining brown with iodine.

Df: deficiency, that is physical loss of an interstitial chromosomal segment.

Cy: Breakage-Fusion-Bridge cycle (written as B.-F.-B. in the text). Thus, Cy(*Bz Wx*) designates a Breakage Fusion Bridge cycle initiated in the region between *Bz* and *Wx*, that is, a kernel showing total loss of *I Sh Bz*, and a mosaic for *Wx-wx*.

E: end; in connection with the above, will be used for the natural distal end of the short arm of chromosome 9.

SFA: Spindle fiber attachment or centromere or kinetochore.

These abbreviations will be used either to designate the class, or directly as a symbol for the number of kernels, or the frequency of kernels observed in that class, depending on the context.

Table 2

Type of test		No. of kernels
$bz/bz \times bz^{m-1}/bz$	pollen 1200 R	780
$bz/bz \times bz^{m-1}/bz^{m-1}$	pollen 1200 R	5005
$bz\ bz \times bz^{m-1}/bz$	ear 1200 R	1265
$bz/bz^{m-1} \times bz/bz$	pollen 1200 R	444
$bz^{m-1}/bz^{m-1} \times bz/bz$	pollen 1200 R	2529
Kernels capable of detecting $bz^{m-1} \rightarrow Bz$		~8778

Results

Tabulation of data. The data under discussion are presented in Table 3. Kernels are arbitrarily classified into 4 size classes, according to the area covered by the loss or aberration pattern. Class 1 consists of kernels whose entire area is affected; class >0·25 of fractionals averaging about $^1/_2$; class 0·25-0·05 average about $^1/_{10}$; the last class <0·05 includes small spots, but not of course the minute losses of a few cells which occur sporadically in controls, and whose frequency is not appreciably raised by radiation.

It is at once apparent that while X-rays produce predominantly but not exclusively whole kernel effects, ultra violet gives rather more fractionals than whole kernels. This is in agreement with the results of Stadler and Sprague (1936) and indeed seems to be rather characteristic of these two mutagens in other material as well; the significance of this will be discussed on another occasion.

Table 3

	Size class	B.-F.-B. cycles starting in				Interstitial losses			End losses				More complex	All affected	Total examined
		E-*I*	*I*-*Sh*	*Sh*-*Bz*	*Bz*-*Wx*	*Sh*	*Sh Wx*	*Wx*	*I*	*I Sh*	*I Sh Bz*	*I Sh Bz Wx*			
U.V.	1	67	7	2	20	4	—	6	2	—	1	59	21	189	
	>0·25	27	5	—	27	—	2	35	23	1	17	112	13	262	
	0·25–0·05	10	1	—	6	—	1	14	14	1	1	26	1	75	
	<0·05	14	1	—	5	—	—	3	15	—	1	49	—	88	
	Total	118	14	2	58	4	3	58	54	2	20	246	35	614	8260
X-ray	1	154	12	4	101	8	3	23	13	6	36	197	61	618	
	>0·25	4	2	—	1	—	—	4	—	—	2	27	—	40	
	0·25–0·05	2	—	—	2	—	—	2	1	—	—	25	—	32	
	<0·05	8	—	—	1	—	—	3	6	—	1	24	—	43	
	Total	168	14	4	105	8	3	32	20	6	39	273	61	733	6782
	General total	286	28	6	163	12	6	90	74	8	59	519	96	1347	15042

It is unlikely that size classes can be assigned without bias, in the same way for all phenotypes. B.-F.-B. cycles are undoubtedly scored somewhat differently from losses. An extreme instance is that no attempt is made to recognize L(*Sh*) affecting part of a kernel. For these reasons, and also because of the extreme irregularity of endosperm development, no great importance can be attached to the size class distribution.

It will be particularly noted that no unambiguously identifiable non-consecutive losses were found: four out of the five possible non consecutive losses involve no confounding and would certainly have been recognized. Two among these four, L(*I Wx*) and L(*I Sh Wx*) taken jointly are free even of any possible doubt associated with *Sh*. L(*Sh Wx*) is given as such in the table, to follow consistently the rule of listing the minimum loss; it is, however, confounded with the consecutive interstitial loss L(*Sh Bz Wx*) and there seems to be no reason to doubt that L(*Sh Wx*) is in fact L(*Sh Bz Wx*).

Statistical examination. At the doses used, X-rays produced 1·45 times more affected kernels than did U.V. The χ^2 for 1 d/f for this difference is 52, so there is no question as to its significance.

We may now examine whether the various types are in proportion, by calculating a heterogeneity χ^2 among the affected losses. This gives a $\chi^2 = 53{\cdot}8$ for 11 d/f, whose P is considerably less than 0·001. If the χ^2 is partitioned into a *within groups* and a *between groups* component, the groups being B.-F.-B cycles, interstitial losses, end losses and more complex, both portions are significant: within classes $\chi^2 = 21{\cdot}8$ (7 d/f) and between classes $\chi^2 = 31{\cdot}9$ for (3 d/f). Examining the heterogeneity *within groups* in more detail, we find:

1. No heterogeneity within B.-F.-B. cycles $\chi^2 = 2{\cdot}2$ for 2 d/f, P = 0·7—0·5 (the two smaller classes being pooled).

2. Heterogeneity within interstitial losses $\chi^2 = 4{\cdot}1$ for 1 d/f, P = 0·05—0·02 (The two smaller classes pooled: this test is perhaps doubtful because of small numbers.)

3. Marked heterogeneity within end losses $\chi^2 = 18{\cdot}1$, 2 d/f (small class pooled).

Much weight cannot be attached to such tests, since they imply that the data within classes are homogeneous, while all experience with irradiated material indicates that this is untrue. Statistical techniques for testing homogeneity in data of this sort involving small frequencies, for example, between ears within classes, are not developed. If the two classes L(*Wx*) and L(*I*), both of which are in relative excess among ultra violet treatments, are omitted, the general heterogeneity diminishes to a non-significant value, $\chi^2 = 14{\cdot}1$ (9 d/f), whose P = 0·2—0·1; since the choice of classes to be omitted depended on their deviation, the assignment of a probability is logically difficult. The small but probably real differences between X-ray and U.V. data will be dealt with later. In the meantime, it seems justifiable to pool them: on the whole, the relative frequencies of various observed classes produced by the two mutagens are remarkably concordant.

In the discussions which follow, it has seemed best to speak in general of the actual numbers of kernels observed, or estimated, in the sample of 15042 rather than percentages. This leads to a more transparent presentation, since an actual

number so given at once conveys, to anyone practiced in examining genetic data, a rough idea of the degree of its reliability. A percentage or proportion would, on the other hand, have to be accompanied by two fiducial limits, a lower and an upper one, since we are dealing with low frequencies and therefore grossly skew distributions. Moreover, such fiducial limits would often be misleading because of undetected but undoubted heterogeneity.

Discussion

General character of the observed effects. It is at once obvious, without any statistical tests, that consecutive multiple losses are much more frequent than the product of the frequencies of the losses of the individual markers involved. For losses of three or four markers, the probability of coincident loss is infinitesimal. This is important as an immediate and direct demonstration that point mutations are not a numerically important ingredient of the data. More evidence in the same direction is provided by the absence of non-consecutive losses: these presumably are occuring at a frequency equal to the product of the frequencies of the losses of the individual markers, unlike the consecutive ones, and so are not observed in a sample of this size. For instance we would expect $L(I) \times L(Wx) = 74 \times 90/15042 = 0{\cdot}04$ kernels of the non-consecutive class $L(I\ Wx)$. These facts show quite conclusively that we are here dealing almost entirely with chromosomal rather than genic phenomena. It is reasonable to suppose that most single marker losses are likewise chromosomal, but there is obviously room for some point effects as well.

Breakage-Fusion-Bridge cycles. These constitute in several respects the simplest group: there cannot be much doubt that nearly all are the immediate effect of a free chromosome end produced by the mutagen. Phenotypically, their detection is very reliable. A few, however, are generated by the breaking of a bridge from more complex aberrations which are not identifiable as such, but a consideration of the more complex class shows that the number must be small. For instance, a dicentric translocation with the break point in E-*I*, and whose first bridge should happen to break distal to *I*, may look like Cy(E *I*). Likewise, a ring with a break in *Bz Wx* may look like Cy(*Bz Wx*). We will not attempt to correct for this: a correction would be complicated to work out, but numerically small. Moreover, it would have no importance for the present purpose, since the relevant part of the more complex event in such cases is also a chromosome break in the same chromosome segment. The partition-subpartition relation at once gives the order of the markers in a B.-F.-B. cycles and only cases where this order was normal are included in the category: other cases go into the *more complex* group. The geometry of the loss pattern occasionally gives an indication as to whether a break was close to, or far from, the first marker involved. This sort of information is not used here.

The numbers of B.-F.-B. cycles generated in the successive marked segments are plotted in Fig. 1. It is immediately apparent that the probability of a B.-F.-B. cycles is proportional to cytological length; within the limits of error, the fit could not have been closer. This fact greatly simplifies further analysis, and permits much more detailed conjectures than would otherwise have been possible.

Cy(*W.x* SFA) are of course not detectable as such, and the basis for estimating their number is given in a separate section.

As a first approximation, we conclude that mutagens produce breaks roughly in proportion to pachytene length. This is essentially in agreement with Longley (1950) who plotted the positions of 1176 breaks occuring in translocations from various ionizing radiation treatments. Longley in his paper emphasizes the discrepancies between length and rate of breakage, and there is no question as to the reality of this. Deeply staining regions near the centromere tend on the whole to have more, and terminal segments fewer breaks than the average. However these discrepancies are not very large, though significant. Few exceed 20%, and most are below 10%. When the errors of absolute pachytene length measurement are considered, as well as sampling errors, one is astonished more by the general agreement than by the discrepancies. The statement that B.-F.-B. cycle breaks occur in proportion to length should be seen in the same perspective: more extensive data may well reveal departures, but they are unlikely to be large. In the short arm of chromosome 9, Longley records only 29 breaks, 13, 10, 6 in three segments going outwards from the centromere; clearly no useful comparison is possible. The breaks recorded by Longley need not be strictly comparable to ours. On the one hand, seeds were irradiated, on the other, a translocation break involves not only the probability of production of a break, but also of reattachment, and the subsequent preservation of translocation is not entirely free of selection factors. In general outline, the picture presented by Kaufmann (1946) for X-ray induced breaks in the X-chromosome of *Drosophila melanogaster* is similar.

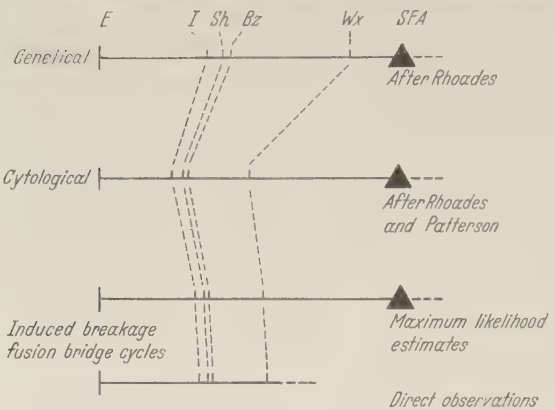


Fig. 1

Breakage-Fusion-Bridge cycles in unmarked chromosome arms. The short arm of chromosome 9 represents about $^1/_{37}$ or 2·7% of the entire chromosome complement at pachytene. In this arm we have 483 observed B.-F.-B. cycles, and a further estimated 384 B.-F.-B. cycles in the unmarked portion, making a total of 867 out of 15042 kernels. In the same sample, we would expect $867 \times 100/2{\cdot}7 = 32111$ B.-F.-B. cycles in the entire irradiated chromosome complement, $32111 - 867 = 31244$ occurring in the 19 unmarked chromosome arms. The assumption that breaks are produced approximately in proportion to chromosome length seems justifiable in the present context. One might prefer not to use the estimate of 384 Cy(*W.x* SFA), but it is clear that a calculation making use of chromosome length alone would give almost the same result. The treatments given result in the production, among all 20 treated chromosome arms, of the surprisingly high average number of $32111/15042 = 2{\cdot}13$ B.-F.-B. *per kernel.*

As an approximation, we may assume that the frequencies of nuclei having 0, 1, 2 ... B.-F.-B. cycles anywhere in the complement are distributed in a Poisson series. This gives:

Nuclei with 0 B.-F.-B. cycles = 11·8%
nuclei with 1 B.-F.-B. cycles = 25·2%
nuclei with 2 B.-F.-B. cycles = 26·9%
nuclei with 3 B.-F.-B. cycles = 19·2%
nuclei with 4 B.-F.-B. cycles = 10·2%
nuclei with 5 B.-F.-B. cycles = 4·4%
nuclei with 6 B.-F.-B. cycles = 1·5%

To keep this calculation as free from theoretical assumptions as possible, observed rather than estimated frequencies of B.-F.-B. cycles are used, except for Cy(*Wx* SFA). It is clear that the conclusions would be almost the same if estimated values, obtained in a later section, were to be substituted. Thus, 88·9% of kernels have one or more B.-F.-B.'s somewhere in the complement, but only 3·2% show them. Similar calculations for the other observed aberrations would yield even more extreme figures, but will not be given since the order of magnitude alone is of importance. There cannot be much doubt that no other experimental material used for studying mutagenesis produces such a high frequency of effect.

Estimate of Breakage-Fusion-Bridge cycles in the interval Wx SFA. There were in the data 519 L(*I Sh Bz Wx*), and it is clear that Cy(*Wx* SFA) will produce this phenotype. The phenotype would also be produced by segmental deficiencies, Df(*I Sh Bz Wx*) having one break in E-*I*, another in *Wx* SFA; and by true terminal losses, if any of these occur, having a break in *Wx SFA*. Segmental deficiencies need not necessarily be simple: some of them may be part of a more complex rearrangement. If we make the simplest possible assumption, that the only numerically important ingredients are segmental deficiencies and B.-F.-B. cycles, and the further assumption that in a given chromosome segment the frequency of breaks giving deficiencies is proportional to the frequency of breaks giving B.-F.-B. cycles, we shall be in a position to calculate the number of these events in the unmarked segment, *Wx* SFA. Moreover, because several kinds of deficiencies are separately identifiable, the calculation involves a constraint or "fix" which verifies its validity in several respects.

To illustrate the principle, we could as a first approximation proceed as follows, the basic data being:

$$\begin{aligned} \mathrm{L}(I\ Sh\ Bz\ Wx) &= 519 \\ \mathrm{L}(Wx) + \mathrm{L}(Sh\ Wx) &= 96 \\ \mathrm{Cy}(\mathrm{E}\ I) &= 286 \\ \mathrm{Cy}(I\ Wx) &= 197 \end{aligned}$$

Let the unknown be Cy(*Wx* SFA). Our initial assumptions can be written in the form of two equations:

$$\mathrm{L}(Wx) + \mathrm{L}(Sh\ Wx) = \mathrm{Df}(Wx) + \mathrm{Df}(Sh\ Wx) = \mathrm{Cy}(I\ Wx) \cdot \mathrm{Cy}(Wx\ \mathrm{SFA}) \cdot \mathrm{K}$$
$$\mathrm{L}(I\ Sh\ Bz\ Wx) = \mathrm{Cy}(\mathrm{E}\ I) \cdot \mathrm{Cy}(Wx\ \mathrm{SFA}) \cdot \mathrm{K} + \mathrm{Cy}(Wx\ \mathrm{SFA}).$$

In addition to the unknown Cy(*Wx* SFA), there is a second auxiliary unknown, the constant K, whose meaning will be discussed later. On solving, we find

Cy(*Wx* SFA) 380. The number of segmental deficiencies contributing to L(*I Sh Bz Wx*) would then be 519—380 = 139.

If these assumptions are correct, we should be able to predict the class L(*I Sh Bz*), a piece of information which has not so far been used, and which is observationally independent of the other data. We have L(*I Sh Bz*) = Df(*I Sh Bz*) Cy(E *I*) · Cy(*Bz Wx*) · K, from which the predicted number of L(*I Sh Bz*) = 67, while 59 were found, a satisfactory fit.

However, we might equally have used this observation of L(*I Sh Bz*) — 59 to calculate a slightly different value of Cy(*Wx* SFA). The real problem then is to combine all the possible ways of finding the unknown Cy(*Wx* SFA) into a single Maximum Likelihood estimate. Because of the complication introduced by the confounding L(*Wx*) with L(*Bz Wx*), it will be preferable, at this point, to state the problem in a simplified general form.

Suppose the chromosome arm is:

E A B SFA

Five frequencies are observed:

$$\begin{aligned} \mathrm{Cy(EA)} &= \alpha \\ \mathrm{Cy(AB)} &= \beta \\ \mathrm{L(A)} &= \mathrm{c} \\ \mathrm{L(B)} &= \mathrm{d} \\ \mathrm{L(AB)} &= \mathrm{e} \end{aligned}$$

Cy(B SFA) = x is the unknown. Our assumptions about the chromosome mechanics by which these classes arise can then be stated as 3 equations:

$$\mathrm{L(A)} = \mathrm{Df(A)} = \mathrm{Cy(EA)} \cdot \mathrm{Cy(AB)} \cdot \mathrm{K} \qquad \text{I}$$

$$\mathrm{L(B)} = \mathrm{Df(B)} = \mathrm{Cy(AB)} \cdot \mathrm{Cy(B\ SFA)} \cdot \mathrm{K} \qquad \text{II}$$

$$\mathrm{L(AB)} = \mathrm{Cy(EA)} \cdot \mathrm{Cy(B\ SFA)} \cdot \mathrm{K} + \mathrm{Cy(B\ SFA)} \qquad \text{III}$$

It is also implied that Df(AB) = Cy(EA) . Cy(B SFA) . K but this last relation contributes no new information. These three equations have but two unknowns. We may take any of the three pairs and obtain three different solutions; in fact, our estimate of 380 Cy(*Wx* SFA) is equivalent to solving the pair: II—III. To make a single best estimate, we note that I, II, and III imply a constraint, or fixed relation between the parameters of which α, β, c, d, e are the observed estimates, being subject of course to binomial sampling error. Let the corresponding Maximum Likelihood estimates be Pα, Pβ, Pc, Pd, Pe. Simple algebraic manipulation of I, II, III shows that

$$\mathrm{Pe} = \frac{\mathrm{P}\alpha\,\mathrm{Pd}}{\mathrm{P}\beta} + \frac{\mathrm{P}\alpha\,\mathrm{Pd}}{\mathrm{Pc}} \qquad \text{IV}$$

and this relation provides the means of making a single Maximum Likelihood estimate of x.

To apply this principle directly to our data involves considerable complications because of the confounding of L(*Wx*) with L(*Bz Wx*) and also the additional

information provided by *Sh*. The only practical way out is to discard a small amount of information, namely Cy(*I Bz*) 34, and in effect to consider the chromosome as though the short segment *I Bz* had been compressed, and thus transformed into the single marker A of the simplified example. We must now estimate Df(*Wx*) from the observed L(*Bz Wx*) + L(*Wx*) = 90. It can be seen that the simplest approximate estimate is

$$90 \cdot \frac{\mathrm{Cy}(Bz\ Wx)}{\mathrm{Cy}(Sh\ Wx)} = 86{\cdot}8$$

Since these corrections are numerically small, and their effect on the final estimate quite trivial, they will not be discussed further.

Substituting the data thus corrected and simplified into IV we get:

$$519 = \frac{286 \times 86{\cdot}8}{163} + \frac{286 \times 86{\cdot}8}{59}$$

It can be seen that the fit is by no means exact, but about as good as could be expected, when it is considered that the numbers are subject to binomial variance, and are all frequencies observed in a sample of 15042.

I am much indebted to Dr. E. Novitski for proposing and obtaining the Maximum Likelihood solution of this problem. The rather lengthy algebra will not be given here; essentially, the procedure consists in obtaining, after substitution for Pe in IV, expressions for the four partial log likelihood derivatives:

$$\frac{\partial \log \mathrm{L}}{\partial \alpha}, \qquad \frac{\partial \log \mathrm{L}}{\partial \mathrm{d}}, \qquad \frac{\partial \log \mathrm{L}}{\partial \mathrm{c}}, \qquad \frac{\partial \log \mathrm{L}}{\partial \beta}.$$

Equating these to 0 gives four simultaneous equations, the first two linear, the second two quadratic, with four unknowns. These are now solved by iteration, giving:

Cy(E *I*)	Pα = 274·5
Cy(*Bz Wx*)	Pβ = 160·3
L(*I Sh Bz*)	Pc = 59·0
L(*Wx*)	Pd = 82·8
L(*I Sh Bz Wx*)	Pe = 525·7

These estimates fulfil equation IV exactly, and, when substituted into I, II, III, give the same answer, whichever pair of simultaneous equations is taken. Thus we obtain the best estimate for Cy(*Wx* SFA) = 384, a value quite close to the provisional partial estimate of 380 given earlier. It is apparent that the additional complexities that would be introduced into this complete solution by taking into account the interval *I*—*Bz* would not be worthwhile.

Thus, while the observed number of L(*I Sh Bz Wx*) is 519, the best estimate is 526, and this consists of 384 Cy(*Wx* SFA) and 142 Df(*I Sh Bz Wx*). The estimate for Cy(*Wx* SFA) corresponds quite well with cytological length: it is slightly, but probably not significantly larger than expected (Fig. 1). If this small excess is real, it may correspond to the excess of breaks reported by Longley (1950) in heterochromatic regions adjacent to centromeres; or it may represent a small contribution from the more complex class, as will be seen later.

The origin of deficiencies. Since we are dealing with fairly long chromosome distances, it seems reasonable to assume the deficiencies are usually of the two-event kind, requiring two separate chromosome breaks for their production. The word event is often used in connection with target theory, and it should be made clear that in the present context there is no such implication. An event is simply a single open break, which may or may not be attributable to some particular target theory phenomenon such as an ionization cluster. When such an event, or free break, occurs alone in the nucleus, three possibilities are open, in principle. The break may:

1. Restitute and become undetectable (except conceivably as a point mutation).
2. Generate a B.-F.-B. cycle.
3. Become a stable terminal deficiency.

Possibility 2 is directly observable, but whether possibility 3 actually occurs in maize endosperm is open to question and will be examined in a special section. Two events in the same chromosome arm may in addition yield either a segmental deficiency or an inversion, and from the frequency of deficiencies it is possible to deduce the minimum number of independently occuring events producing them.

Such an estimate is necessarily an absolute minimum, for it implies the quite unrealistic assumption that whenever two events occur in the same chromosome arm the result is invariably a deficiency. It is obvious that if single breaks often restitute, the same can happen with more than one; however, an estimate of the minimum number of events is not without interest and the artificial assumption that the piece of chromatin between two events is always lost is made to permit this.

If there are two adjacent segments

$$A \qquad l_x \qquad B \qquad l_y \qquad C$$

of lengths l_x, l_y respectively, measured in units proportional to break frequency. then the assumptions made amount to

$$Df\,(B) = 2\,xy$$

which can be solved by taking into account $x = y\,\frac{l_x}{l_y}$, x and y being the frequency of events in the two segments. At the same time, there will be x^2, y^2, undetectable deficiencies within the segments l_x, l_y, respectively. If there are more than two adjacent segments it becomes possible to estimate the minimum number of events without making use of any independent measures of length. Thus, for 3 segments we have:

$$A \qquad l_x \qquad B \qquad l_y \qquad C \qquad l_z \qquad D$$

$$Df(B) = 2\,x\,y, \qquad Df(C) = 2\,y\,z, \qquad Df(BC) = 2\,x\,z,$$

giving 3 equations for the 3 unknowns x, y, z. Here again, there will be x^2, y^2, z^2 undetectable deficiencies within the segments l_x, l_y, l_z.

These relations hold if events are independant of each other, so that the frequencies of 0, 1, 2, ... events in a given segment are in a Poisson distribution. Departures from independence may arise in many ways which are physically diverse, but statistically equivalent and experimentally indistinguishable. For example the same ionizing particle may cause two breaks in a proportion of cases, or part of the sample may have received a higher dose than another part. While there is no doubt that heterogeneity exists in our data, it is hoped not to be large enough seriously to vitiate the proposed estimate.

In the Maximum Likelihood estimate of Cy(*Wx* SFA), deficiencies were equated to the product of B.-F.-B. cycles in the adjacent sections, multiplied by a constant K. If we put $m = \sqrt{\frac{1}{2}K}$, this can be written as:

$$\mathrm{Df(B)} = 2\,\mathrm{Cy(AB)\,m} \times \mathrm{Cy(BC)\,m}.$$

In solving equations I, II, III for x, a Maximum Likelihood solution for K was obtained at the same time; K = 20·156, hence m = 3·174. The minimum number of deficiency generating events is then given by multiplying the number of B.-F.-B. cycles by m. This Maximum Likelihood estimate makes use of all the data used in calculating Cy(*Wx* SFA), by far the greater part of the available information. Data on L(*I*), L(*I Sh*), L(*Sh*), and L(*Sh Wx*) have not so far been used: a single maximum likelihood estimate including these also is possible in principle, but seems to lead to a most unattractive calculation, which will not be attempted. Data on L(*I*) are anomalous on any theory: it is the only case of disproportionality to length, and moreover, shows a discrepancy between the two kinds of radiation. Data on the three other classes of deficiency are numerically so small that they could only contribute little additional information.

Estimate of the minimum frequency of events generating deficiencies. It seems best to use here the estimated rather than the observed frequencies of B.-F.-B. cycles, although the difference between the two is trivial. Since there is no estimate for Cy(*I Sh*) and Cy(*Sh Bz*), the observed numbers are substituted. The number of B.-F.-B. cycles is given at the left of Table 4 and the corresponding

Table 4

B.-F.-B. cycles		E	*I*	*Sh*	*Bz*	*Wx*	SFA
		870	89	19	508	1219	
	E		(*I*) 20·6	(*I Sh*) 4·4	(*I Sh Bz*) 58·8	(*I Sh Bz Wx*) 141·0	
274	*I*	50·3		(*Sh*) 0·2	(*Sh Bz*) 6·6	(*Sh Bz Wx*) 15·9	
28	*Sh*		0·5		(*Bz*) 1·3	(*Bz Wx*) 3·1	
6	*Bz*			0·02		(*Wx*) 82·3	
160	*Wx*				17·1		98·8
384	SFA						

Total minimum deficiency generating events: 2705. Total deficiencies, detectable and undetectable: 501. All entries are for a total of 15042 kernels.

minimum number of events along the upper margin, while the resulting deficiencies are entered in the body of the table. The undetectable deficiencies within segments are in the diagonal.

It must be emphasized that this estimate, even as a minimum, is only intended for an approximation. The theory on which it is founded is an oversimplification: moreover, even the statistical accuracy of K, and hence m, is not high. Still cruder, but almost independent estimates are possible which make no use of any measure of length such as B.-F.-B. cycles, but instead, compare deficiencies generated in three adjacent regions, as was explained earlier. They are of some value as verifications of the validity of the more complete estimation, but will not be presented in detail.

Table 5

Class	Number observed	Calculated from products of minimum estimated events, from table
L(*I*)	74	20·6 Df(*I*)
L(*I Sh*)	8	4·4 Df(*I Sh*)
L(*I Sh Bz*)	59	58·8 Df(*I Sh Bz*)
Df(*I Sh Bz Wx*) (estimated)	142	141·0 Df(*I Sh Bz Wx*)
L(*Sh*)	12	6·8 Df(*Sh*) + Df(*Sh Bz*)
L(*Sh Wx*)	6	15·9 Df(*Sh Bz Wx*)
L(*Wx*)	90	85·4 Df(*Wx*) + Df(*Bz Wx*)

All numbers are for a sample of 15042.

The triplet L(*Sh*), L (*Sh Wx*), L(*Wx*) gives a total of 1823 events for the interval *I*-SFA, as compared with 1835 by the method using m, a very close fit, which is largely fortuitous when the small numbers used are considered. The other usable triplet L(*I*), L(*Sh*), L(*I Sh Bz*) gives a total of 2258 events for E-*Wx*, as compared with 1486 by the method using m. It involves the aberrant class L(*I*) but is still of the right order of magnitude. In both of these estimates, the rather unsatisfactory class L(*Sh*) has to be used.

There is very little point in presenting a map of the chromosome arm in deficiency generating event units. Since these events are estimated as the product m × (estimated B.-F.-B. cycles) such a map, if plotted, would be closely proportional to map of observed B.-F.-B. cycles given in Fig. 1, for observed and estimated B.-F.-B. cycles coincide very well. In fact, the maps would differ essentially only by the proportionality constant m.

A more interesting check on the calculation is to compare the frequency of observed deficiencies with the frequencies predicted by multiplying together deficiency generating events in the relevant segments. The data are already included in Table 4 but are presented in more convenient form for comparison in Table 5. A very satisfactory fit is observed, the only discrepancy being the anomalous class L(*I*) which has already been discussed.

Tables 4 and 5 present in condensed form the evidence for the proposed mechanism of the origin of deficiencies. To the extent that a satisfactory fit is observed, Table 5 can be taken as strong evidence that the proposed mechanism is consistent with the facts and capable of accounting for observations. Given a checkerboard such as that of Table 4, with observations entered into those cells that correspond to recognizable deficiencies, it is not possible to find marginal

entries (events) which will give products which fit any *arbitrary* set of observations. It is only if the classes of deficiencies have definite relations to one another that a consistent set of marginal entries can be found. Thus, the fact that a consistent set of marginal entries is possible at all is in itself evidence that the proposed relations between deficiency classes are well founded. When it is found that the set of marginal entries in fact also corresponds to the best measure of chromosome length in breakage units, the case seems to be decisive. Because of the complex constraints imposed by the mechanism on the proportions of the various classes, data of this kind yield much more precise information than the relatively low statistical accuracy of the individual numbers would lead one to believe.

The numbers given are Maximum Likelihood estimates giving the best possible fit, with a few restrictions and complications discussed earlier, and presumably present the truest picture. But it is worth pointing out that essentially the same conclusions can be arrived at by much simpler and cruder considerations, although with greatly reduced accuracy. We can simply multiply actual cytological lengths together, as measured on pachytene preparations, and then multiply them by the ratio of the totals, to make total observed deficiencies equal the total predicted. Using the Rhoades-Patterson cytological map, one gets (see Table 6). Cytological data are not very accurate, and thus, the fit is not as good as before, there being two or three fairly large discrepancies; the discrepancies are, however, of the same order as the differences between several available cytological maps and the largest discrepancy, as in the exact calculation, is for Df(*I*). In general, the picture is about the same as when Maximum Likelihood estimates are used.

Table 6

Observed	Predicted
74	10·9 Df(*I*)
8	5·4 Df(*I Sh*)
59	59·8 Df(*I Sh Bz*)
142 (estimate)	146·8 Df(*I Sh Bz Wx*)
12	10·0 Df(*Sh*) + Df(*Sh Bz*)
6	22·6 Df(*Sh Bz Wx*)
90	135·5 Df(*Wx*) + Df(*Bz Wx*)

In summary, we conclude that deficiencies are in fact simple two event segmental deletions, in the great majority of cases. There is no reason to suppose that special one-event erasures of gene loci make any appreciable contribution, or that any other phenomena are occuring. An additional fact is also brought out, that the probability of production of a deficiency is independent of its length. Large and small deficiencies are equally likely, the only elements that determine their frequency being the lengths of the segments in which breaks must occur. This last conjecture must of course be taken in its proper context, and with due regard to sampling error. More data may always reveal small differences not at present demonstrable, and tests on special classes of deficiencies like the minutes of *Drosophila*, may give different conclusions.

True stable terminal deficiencies. So far the analysis has been carried out as though all *end loss* phenotypes are produced by interstitial deficiencies, with one break in the region E-*I*, and as though all simple single breaks generate B.-F.-B. cycles. The assertion that true stable terminal deficiencies can be produced in maize endosperm, particularly by ultra violet, has been made on a number of

occasions in the literature from superficial appearances, but critical observations have been lacking. We shall now examine this possibility.

If we have two markers on a chromosome arm,

$$\underset{|}{E} \quad l_x \quad A \quad l_y \quad \underset{|}{B} \quad l_z \quad SFA$$

we can compare the frequency of L(A) with L(B), l_x, l_y, l_z being as before, chromosome lengths in units proportional to rate of breakage. If both classes are made up entirely of interstitial deletions, one would expect:

$$\frac{L(A)}{L(B)} = \frac{l_x\, l_y}{l_y\, l_z} \quad \frac{l_x}{l_z}$$

Several instances where this simple consideration can be applied would not, individually, give worthwhile sensitivity because of small numbers. In the case of L(*I*) moreover, some departure from proportionality of rate breakage to length is indicated. While Df(*Wx*) is confounded with Df(*Bz Wx*), we have seen that it can be estimated as a first approximation as:

$$Df\,(Wx) = \frac{L(Wx)\,Cy\,(Bz\ Wx)}{Cy\,(Sh\ Wx)} = 86 \cdot 8$$

which would lead to

$$\frac{Df(Wx)}{Df(I\ Sh\ Bz)} = \frac{86{\cdot}8}{59} = \frac{Wx - SFA}{E - I}$$

This is clearly approximately true, on whatever basis distance is estimated. On the other hand, if a large proportion of L(*I Sh Bz*) were to consist of stable terminal deficiencies, an intolerable discrepancy would appear. For instance, if $^1/_2$ of L(*I Sh Bz*) were true terminal, we would have $86{\cdot}8/^1/_2(59)$ for the chromatin length ratio *Wx*—SFA/E-*I*, which conflicts with the facts. The case is not changed if another estimate of Df(*Wx*), such as 82·3 of the preceding section is used. In none of the other instances does a better fit ever result from assuming the presence of stable terminal deficiencies.

It is possible to combine all these individual cases into a single comprehensive test, in which we shall attempt to estimate the maximum number of stable terminal deficiencies that could be present without doing violence to the data.

Suppose that apparent terminal losses consist of some interstitial deficiencies, Df, and in addition some stable terminal deficiencies whose frequency is T(Cy). This additional unknown T can now be introduced into equations I, II, and III (p. 404) as a factor by which the frequency of B.-F.-B. in a given segment is multiplied to get the frequency of stable terminal deficiencies arising in that segment. The equations then become:

$$\begin{aligned} c &= \beta(\alpha K' + T) \\ d &= \beta K' x' \\ e &= (\alpha K' + T + 1)\, x' \end{aligned}$$

The meaning of the other symbols being as before. Solving for the three unknowns gives:

$$\begin{aligned} x' &= \quad 0{\cdot}0253335 \\ K' &= \quad 21{\cdot}0203 \\ T &= -0{\cdot}0376979 \end{aligned}$$

It will be seen that x′ is almost the same as x, and K′ as K. T is a small negative number, so that the best fit is obtained by assuming that there are no stable terminal deficiencies.

We now need the fiducial limits of T, the largest positive value T might have with a probability of, say, 0·05. The general Log. Likelihood expression is:

$$L = 86{\cdot}8 \log K' + 605{\cdot}8 \log x' + 59 \log (\alpha K' + T) + 519 \log (\alpha K' + T + 1) + \\ + \log [1 - \alpha - \beta - \beta (\alpha K' + T) - \beta x' K' - x' (\alpha K' + T + 1)]$$

equating the expressions for:

$$\frac{\partial L}{\partial x'}, \quad \frac{\partial L}{\partial K'}, \quad \frac{\partial L}{\partial T}$$

respectively to 0, and solving, one obtains, of course, the values for x′, K′, T given above, a procedure for which there is no need since we have three equations with three unknowns, an exact fit and no estimation problem. An information matrix is now formed of the six partial second derivatives:

$$I = \begin{bmatrix} \frac{\partial^2 L}{\partial x'^2} & \frac{\partial^2 L}{\partial x' K'} & \frac{\partial^2 L}{\partial x' T} \\ \frac{\partial^2 L}{\partial x' K'} & \frac{\partial^2 L}{\partial K'^2} & \frac{\partial^2 L}{\partial K' T} \\ \frac{\partial^2 L}{\partial x' T} & \frac{\partial^2 L}{\partial K' T} & \frac{\partial^2 L}{\partial T^2} \end{bmatrix}$$

which, after change of sign and inversion, yields the coinformation matrix:

$$I^{-1} = \begin{bmatrix} + 0{\cdot}000001981 & - 0{\cdot}001671 & - 0{\cdot}000010004 \\ - 0{\cdot}001671 & + 6{\cdot}469 & - 0{\cdot}08885 \\ - 0{\cdot}000010004 & - 0{\cdot}08885 & + 0{\cdot}003252 \end{bmatrix}$$

The diagonal terms of this matrix are the required variances. The basis for this procedure is given, for example, by Rao (1952). In particular, the standard error of T is

$$\sqrt{0{\cdot}003252} = 0{\cdot}05703;$$

whence the fiducial limit of T at the 1 in 20 level is 0·1118. Thus, T does not differ significantly from 0, and if T were in fact 0·1118, then only once in twenty trials would a sample of the kind observed or more extreme have occurred.

Directly observed end losses L(*I*), L(*I Sh*), L(*I Sh Bz*), L(*I Sh Bz Wx*) total 660. Stable terminal losses simulating these could arise in the chromosome segment I—SFA, a segment in which we have, in the successive marked regions going from *I* to the centromere, $28 + 6 + 163 + 381 - 578$ B.-F.-B. cycles, the first three being direct observations, the fourth an estimate. For this estimate of 381 we are using x′ rather than x, but the difference this makes is trivial. Within the estimated fiducial limits, there could be $578 \times 0{\cdot}1118 - 65$ stable terminal deletions, among the 660 observed end losses. It should be particularly noted that x′ is very nearly the same as x, and K′ as K, as without this circumstance there would be a logical difficulty in getting a fiducial limit from an estimation of several parameters simultaneously.

In general, this seems to be a fairly reliable conclusion, with a wide inductive basis; but it should not be taken as a very accurate estimate. In spite of the statistical elaboration, several approximations are involved, but a consideration of them only reinforces the conclusion. While the class of more complex aberrations has been completely disregarded in this calculation, an allowance for their effect makes the case against the existence of stable terminal losses even stronger. One class of symmetrical incomplete two-event exchanges undoubtedly makes a small contribution to the apparent stable end losses. They are the exact equivalent of segmental deficiencies, except that the unmarked terminal piece is not the natural end of chromosome 9, but is some other chromosome arm. Thus, within the already small fiducial margin left for stable terminal end losses, one has to accomodate a few losses due to incomplete exchange. Although the data used in the calculation are entirely consistent, the discrepancy of $L(I)$ shows that phenomena not taken into account may be occurring. In calculating the fiducial limit, a normal distribution is used under circumstances in which this can only be a crude approximation.

However, the following conclusion would seem reliable: the best estimate for the frequency of stable terminal deficiencies is that they do not occur at all. The highest value still compatible with the data, at the 1 in 20 level of probability, is that about 10% of observed end losses are in fact produced by stable terminal deficiencies.

It has been demonstrated by McClintock (1951, and elsewhere) that free, unstable chromosome ends will normally stabilize if passed through a zygotic generation, and there have been indications that the capacity to stabilize may be under genetic control. In view of this, it should not be assumed that the behavior of free ends even in endosperm is necessarily uniform in all material.

More complex aberrations. This class is not very important numerically, 96 kernels only being so classified. An adequate enumeration of all rearrangements that could reasonably contribute to this class is lengthy and complicated, and will not be given; it would be out of proportion to the amount of information that can be extracted from the data, which consist of small numbers, often involving ambiguities of recognition. A very sketchy treatment only will be attempted, aimed chiefly at establishing principles, orders of magnitude, and the degree to which the existence of the more complex class affects the preceeding analysis. Estimates will be derived from models, based on the assumption that induced breaks are in proportion to length, length being measured in B.-F.-B. cycle frequency. It seems pointless to overburden such a treatment with sampling error considerations; it is understood that whenever, for example, a class is said to be equal to another, this means equal within fiducial limits, which in practice may be large.

In speaking of estimates in connection with deficiencies and B.-F.-B. cycles on the one hand, and in connection with more complex aberrations on the other, it should be made clear that the word *estimate* is used in two different senses. In the first case, the basic assumptions can usually be stated with some degree of completeness, and limits of uncertainty can often be established. With more complex aberrations on the other hand, the complexities and uncertainties are

of an entirely different order, and the data themselves small and often ambiguous: in this case, the estimates are to be taken as speculations.

A marker order is only ascertainable if a B.-F.-B. cycle is generated: a departure from the normal partition-subpartition relationship in the mosaic then indicates an aberration with a change of sequence. To be detectable, an aberration must either be *incomplete*, one of the *induced breaks* remaining free, or else a free end may follow the mechanical breaking of the bridge of a dicentric. Thus, with the exception of rings, the aberrations detected in mosaics are of the type resulting in dominant lethals in many other situations; all of these aberrations are of course well known from *Tradescantia*.

While there is usually no difficulty in deciding that an aberration belongs to the more complex aberration class, there is very often ambiguity in assigning it to a subdivision of that class. These difficulties have several distinct origins. Enough mosaic area must be available to establish all the relations between markers; if a B.-F.-B. cycle breaks early in such a way as to lose many or all the markers, an uncertainty results. The place where the first bridges of a dicentric break may also determine whether the aberration is detectable as such or not. Superimposed on these are occasional difficulties of confounding and of the recognition of *sh* discussed earlier. Many of the more complex kernels have to be listed individually, with a separate discussion of the ambiguities and possible interpretations of each. There is one exception to this: a phenotype consisting of a B.-F.-B. cycle for *Wx* without loss of *I*; this is always recognizable and is ascribable to a definite mechanism.

Aberrations can involve any number of chromosome breaks, and experience with *Drosophila* shows that their relative frequencies are far from predictable from simple considerations of probability (e.g. Catcheside 1948). The observation that non-consecutive losses do not occur lead one to infer fairly directly and satisfactorily, that four-event aberrations other than deficiencies would be equally rare. This is justifiable of four breaks in the short arm of chromosome 9, and is believed to be also approximately true for four event aberrations with some of the breaks elsewhere, in the complement, the greater length of chromatin being approximatly compensated by a strong bias against interchromosomal exchange. A few mosaic patterns that could only be produced by aberrations with at least three breaks were seen: only about four such kernels seem to present no ambiguity of interpretation. We shall therefore proceed on the simplifying assumption that aberrations of more than two breaks seem quantitatively unimportant, and may be disregarded in a preliminary survey.

At several points it becomes desirable to know the proportion of breaks that fail to rejoin, or the proportion of incomplete aberrations. There seems to be no entirely satisfactory way to estimate this, but it is possible to place an absolute upper limit for unrejoined breaks associated with deficiencies. The quite improbable assumption can be made that all observed B.-F.-B. cycles are the result of incomplete deficiencies, and never of any of the several other mechanisms discussed such as single unrejoined free breaks or incomplete aberrations of other kinds. To avoid the use of estimated Cy(*Wx* SFA) we consider only L(*I*), L(*I Sh*), L(*I Sh Bz*), L(*Sh*), a total of 153; in addition, there are 67 undetected

but rather reliably inferred deficiencies between markers in the same regions, giving a total of 220. The relevant number of B.-F.-B. cycles is 483. It would then be impossible to have more than $483/(483+220)=69\%$ of unrejoined breaks. This is in no sense an estimate but an extreme upper limit; also, it does not follow that the proportion of free breaks is necessarily the same among potential deficiencies as among potential exchanges. If more plausible assumptions are made, one is led to a value of perhaps about 10%, and for purposes of illustration, this will occasionally be taken.

Two-event aberrations contributing to the more complex class can be considered under five headings: 1. incomplete symmetrical exchanges; 2. incomplete pericentric inversions; 3. incomplete apocentric (= paracentric) inversions; 4. asymmetric exchanges; 5. rings.

The first two classes, incomplete symmetrical exchanges and incomplete pericentric inversions, produce indistinguishable effects and will be considered jointly. A distal part of the short arm of chromosome 9 is translocated onto some other chromosome in case 1, or onto the long arm of chromosome 9 in case 2; in both cases, the proximal part of chromosome 9 remains open to generate a B.-F.-B. cycle. The characteristic phenotype mentioned earlier consisting of a B.-F.-B. cycle mosaic for *Wx*, without loss of *I* is presumably generated in this way; if classification for *Sh* be disregarded, its recognition is very reliable. A total of 17 such kernels were found among the 96 more complex, 14 from X-ray and 3 from ultra violet data. Some deductions from this are possible, but it must be pointed out that the error of such a single small number is large, the P 0·05 fiducial limits being 10 and 27. To produce this phenotype, a break must occur in *I-Wx*, or 0·23 of the entire short arm; whence one concludes that there were $17/0{\cdot}23=74$ in this entire subclass. Among these 74 there must be $74\times0{\cdot}32=24$ Cy(E *I*) and $74\times0{\cdot}45=33$ invisible Cy(*Wx* SFA). The B.-F.-B. cycles are of course already included under the observed and estimated headings of that section. The entire symmetric exchange class, of both translocations and pericentric inversions, can be represented as follows:

		Break in short arm of chromosome 9	
		closed	open
Break in some other arm	closed	Invisible subclass of complete exchanges	Subclass giving the characteristic pattern
	open	Subclass of simple end losses	Subclass of simple B.-F.-B. cycles

It can be seen that whatever the proportion of breaks remaining open, the subclass of simple end losses equals the characteristic subclass. Again, on a basis of chromatin length, $74\times0{\cdot}68=50$ detectable end losses are expected, and $74\times0{\cdot}32=24$ invisible losses with a break in E-*I*, adding 24 undetected events. Besides that of proportionality to length, the only other assumption is that the probability of remaining open is the same for both breaks, and that these are independant. The subclass of simple B.-F.-B. cycles in which both breaks are open, is not a class of exchanges at all but merely of free ends which happen to be ascertained through the subclass of incomplete symmetric exchanges.

To estimate the size of the entire symmetric class, as represented above, requires a knowledge of the proportion of breaks remaining open. If this proportion were in fact 0·1, as was discussed earlier, the entire class would number 822, among which would be 666 invisible complete translocations and 8 B.-F.-B. cycles. Thus, the original observation of 17 kernels leads one to infer $17 + 24 + 666 = 707$ events in the short arm of chromosome 9.

Incomplete apocentric (= paracentric) inversions can yield a recognizable pattern, namely a B.-F.-B. cycle with reversal of markers: unfortunately, in practice it can be confused with the pattern of dicentrics or of rings. For this reason it is difficult to assign kernels to this class with certainty, even though it is felt that a majority of the 79 more complex kernels belonged to this class. Six combinations of two breaks, followed by inversion can yield a recognizable pattern, if the distal break remains open, but two of these six depend on a classification for *Sh*, are doubtful and presumably are numerically unimportant. The four good cases are: (E *I*) (*Bz Wx*): (E *I*) (*Wx* SFA): (*I Sh*) (*Wx* SFA); (*Sh Bz*) (*Wx* SFA): jointly, they are 0·44 of the subclass characterized by an open distal break. The remainder of this subclass, 0·56, consists of 0·36 B.-F.-B. cycles and 0·20 L(*I Sh Bz Wx*). Another subclass, equal to the preceeding and with the proximal break open, would produce 0·55 of various B.-F.-B. cycles and 0·45 of L(*I Sh Bz Wx*). To estimate the remaining two subclasses, 1. in which both breaks are closed and 2. in which both breaks are open, requires a knowledge of the proportion of breaks open. If both breaks are closed, an undetectable deficiency results, while if both breaks are open, a B.-F.-B. cycle is produced. As an illustration, it might be taken that there were 40 kernels showing the characteristic pattern: this is almost certainly of the right order of magnitude. We might further suppose that 0·1 of breaks stay open. We would then have 801 complete inversions, 103 B.-F.-B. cycles and 45 L(*I Sh Bz Wx*); altogether, this implies 1850 necessary undetected events in the short arm of chromosome 9.

Each inversion within the short arm of chromosome 9 requires two breaks, and by "necessary" events are meant breaks occuring thus in pairs. Since events occur independently of one another, there will be many occasions when only one event is produced, a consideration implicit in the treatment that has been given earlier of deficiencies. Among apocentric inversions, there would be implied, in the example given here, a total of 989 *pairs* of events, or $15042 \times 989/15042 = 3857$ events altogether. Of these, $3857 - (2 \times 989) = 1879$ would be occuring singly.

It is the general experience in *Drosophila melanogaster*, that about as many inversions are induced as deficiencies of corresponding size, minutes being disregarded (e.g. Catcheside 1948). This seems to be purely an empirical observation, and there does not appear to be any fundamental reason for such a connection. However, it is worth pointing out that the estimated 801 complete inversions within the short arm of chromosome 9, arrived at by a most tenuous argument more as an example than an estimate, are of the same order of magnitude as the 501 deficiencies estimated on much more reliable grounds earlier. Thus, there are reasons for thinking that the example is not unrealistic. It would be possible to assume that inversions in fact equal deficiencies, say 500 of each, and from this relation, to deduce a proportion of incomplete aberrations a little

greater than 0·1, but the basis for such a calculation seems much too uncertain to justify it.

If the asymmetric translocations class be represented by a diagram similar to that used for the symmetric class, it will be seen that, quite unlike this last. most asymmetrics produce visible effects. If the break adjacent to the centromere of chromosome 9 reunites, a dicentric results, while if it fails to unite, a B.-F.-B. cycle is produced. Among dicentrics, 0·36 are capable of giving a characteristic pattern, most of them (0·32) without loss. It is difficult to say exactly how many of these 0·36 would actually be recognized, as not enough is known about the detailed mechanics of dicentrics, the prefered position of breakage of the bridges, and whether the two bridges tend to break at the same level. However, it would seem likely that a dicentric is most often recognizable. In 0·19 there would be a Cy(*Bz Wx*) whichever way the bridge broke, since *Wx* is then the only marker on the bridge. This can be summarized as:

	Break adjacent to centromere of chromosome 9:	Expectation:
Total asymmetric exchanges and potential exchanges	closed	0·32 mostly recognizable 0·04 various B.-F.-B. cycles 0·19 Cy(*Bz Wx*) 0·45 L(*I Sh Bz Wx*)
	open	0·55 various B.-F.-B. cycles 0·45 L(*I Sh Bz Wx*)

It is difficult to apply this analysis because of confusion of the characteristic class with rings and possible other more complex patterns. More as a plausible illustration than an estimate, we might suppose there were 20 such kernels. This would lead to a total for the closed subclass, of about 70, and, if 0·1 breaks remain open, 8 in the open subclass. It is clear that if any plausible numbers are substituted the total number of asymmetrics will always be much less than of symmetrics.

Now it seems most likely on *a priori* grounds that the number of symmetric exchanges should equal the number of asymmetrics; this is simply an expression of the lack of polarity of chromosomes in rejoining, and observations confirm this for *Tradescantia*. We believe that the explanation lies in the fact that the symmetric consists of both inter and intrachromosomal elements, while the asymmetric class is purely interchromosomal. One might then infer, to use the figures from the example, that the 822 symmetrics consisted of about 78 translocations and potential translocations, and about 744 inversions and potential inversions. This would indicate a strong bias favoring exchanges within a chromosome over those between chromosomes. A bias of the order of 10 to 1 in favor of exchange within the chromosome represents a much greater bias on a random exchange basis. The chromatin length available for interchange with the short arm of chromosome 9 is about 18 times greater among other chromosomes than it is in the long arm of chromosome 9. Thus the true bias would be of the order of 180. The same sort of bias has been demonstrated in other material, an extreme example being female *Drosophila melanogaster* which yields almost no translocations at all. While the argument used to reach this conclusion is indirect and uses many very uncertain steps, it is easy to satisfy oneself

that the final result would be substantially the same even with considerable variations of the initial assumptions.

Ring chromosome behavior has been previously studied by McClintock (1938), but in practice, distinction of the pattern from some of the other more complex aberrations may be doubtful. At least two markers on the ring are needed; like the dicentric, the ring is by definition a complete aberration, the corresponding incomplete aberration being just a pair of free ends, one of which will generate a B.-F.-B. cycle with markers. It would be expected that 0·36 of rings include more than one marker and would be mostly recognizable as belonging to the more complex class. Another 0·19 will carry *Wx* only and be classed as Cy(*Bz Wx*), and 0·45 will produce L(*I Sh Bz Wx*). There cannot be much doubt that rings are a minority among the 79 more complex kernels which could include them. Thus, rings are much less numerous, on a basis of chromosome length, than for instance, deficiencies. Whether this represents a bias against reunion between ends in different chromosome arms, or a difficulty in forming a ring configuration, it is impossible to say.

With very different degrees of uncertainty, the various more complex classes yield about 325 B.-F.-B. cycles, out of the total of 852. The fact that over a third of this class may thus be associated with more complex aberrations in no way invalidates the calculations given earlier in connection with B.-F.-B. cycles. Every time a simple B.-F.-B. cycle is observed, an induced break is infered in a certain position of the chromosome; this in itself is sufficient, and is all that is implied. The fact that other breaks may also be present, is irrelevant in this context.

B.-F.-B. cycles originating mechanically, as the consequence of the breaking of a dicentric or ring, are in a different class, and their presence could seriously affect the calculations. Fortunately, it seems certain that they are numerically infrequent enough to be disregarded.

Restitution. It is of considerable interest to attempt to estimate what proportion of breaks rejoin in their original positions, or restitute, without leaving any trace of the break. Whenever such estimates have been made in other material, extremely indirect arguments have been used. The estimate that seems possible from endosperm mosaics is at least as indirect as any other, but the considerations involved are entirely different. It is interesting to find, for this reason, that one arrives at an estimate of about the same order as has been reported for *Tradescantia*. From the 501 deficiencies of all types, mostly observed or fairly reliably infered, it was deduced that a minimum of 2705 deficiency generating events had occured. Of these, 1002 are actually in the deficiencies, and $2705 - 1002 = 1703$ are single breaks. On much more hypothetical grounds, something like 1879 single events were ascertained from incomplete inversions within the short arm of chromosome 9. It seems much more difficult to estimate single events occuring in the other more complex classes, in which one break is elsewhere than in the short arm of chromosome 9; but their contribution does not seem to be very large. The same is true of rings. Since a minimum estimate will be attempted, $1703 + 1879 = 3582$ events occuring singly may be taken.

Among the 852 B.-F.-B. cycles, about 325 were attributable to the more complex classes, in which they are part of some rearrangement, but are

not observationally distinguishable from single B.-F.-B. cycles. This leaves 852 — 325 = 527 B.-F.-B. cycles which can reasonably be ascribed to single breaks; a few more could perhaps be deducted for dicentric configurations.

Thus, a minimum of 3582 single breaks were accompanied by 527 single event B.-F.-B. cycles, leaving 3582 − 527 = 3055 events which disappeared without trace, or restituted. One might conclude then, that about 3055/3582 = 0·85 of single breaks restituted. There is of course every reason to think that this estimate is in fact too low, and that the amount of restitution is really much greater. It should be pointed out that the supposed 1879 events estimated from incomplete inversions and used in the above calculation, constitute a very speculative figure. However, it is difficult to see how it could be less than half that number, and that will not change the order of magnitude of the estimate. Many assumptions have been made during the argument which all tend to make the amount of restitution look less than it really is.

There is no reason to suppose that the amount of restitution is similar in different organisms; it is also known that some agents, such as α-particles, yield significantly less restitution in *Tradescantia* than do other mutagens. However, it is worth observing that Lea (1946) estimated the proportion of breaks restitution in X-rayed *Tradescantia* as of the order of 0·9, by a variety of methods, with which our estimate agrees rather closely. It should not be assumed that the amount of restitution is independant of the number of breaks initially induced; special experiments are needed to settle this question.

Summary

1. Maize pollen carrying the 9th chromosome dominant markers *I Sh Bz Wx* was irradiated, and used to pollinate plants of the constitution *C sh bz wx*. The treatments used were X-rays, and ultra violet of wavelength 2537 Å., and the resulting endosperm loss and mosaic patterns studied. 15042 kernels were examined, yielding 1347 affected endosperms of all types from all treatments.

2. The basic principles of interpretation of these patterns in terms of the underlying chromosome aberrations are discussed in detail.

3. It is characteristic of this material that a free broken chromosome end generates a Breakage-Fusion-Bridge cycle which typically yields a recognizable pattern and permits the determinations of a marker gene sequence.

4. The basic induced aberrations detectable are 1. directly induced free ends; 2. free ends resulting from dicentrics or rings; 3. segmental deficiencies; 4. incomplete aberrations of various kinds.

5. Radiation produces Breakage-Fusion-Bridge cycle generating breaks at a rate very closely proportional to pachytene chromosome length.

6. All data are consistently explained by assuming that breaks yielding other aberrations, and particularly deficiencies, are likewise produced in proportion to length.

7. Stable free chromosome ends most likely do not occur at all in maize endosperm, as a result of either X-rays or ultra violet. If they do, it can only be infrequently and their quantitative contribution can only be small. Fiducial limits for this are calculated by a Maximum Likelihood method.

8. There is no reason to suppose, that point effects make any significant contribution.

9. Probably all, and certainly the great majority, of stable losses of markers are due to simple segmental deficiencies produced by two chromosome breaks. The minimum frequency of such deficiency generating events is estimated.

10. By an application of Maximum Likelihood, the number of Breakage-Fusion-Bridge cycles in the unmarked region between *ux* and the centromere is estimated. These breaks are also closely proportional to pachytene length.

11. More complex aberrations cannot be handled as satisfactorily as Breakage-Fusion-Bridge cycles or deficiencies, but some conclusions about them are possible. There are indirect indications of a strong bias, possibly of the order of $\times 100$ or $\times 200$ favoring rearrangements within a chromosome against those between chromosomes.

12. An indirect argument leads to the estimate that a minimum of 0·85 of all breaks restitute without trace.

13. Separate tests indicate that the numerous breaks induced by radiation throughout the chromosome complement do not generate *de novo* genes having the properties of *Ac*, on the scale of these experiments.

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ANTIGENIC VARIATION IN *PARAMECIUM AURELIA*, VARIETY 9

By

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With 3 figures in the text

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Introduction

A ninth variety (or physiological sub-species) of *Paramecium aurelia* has recently been discovered (Beale and Schneller 1954), and found to be the commonest of the varieties occurring in Scotland. Some stocks of variety 9 have also been found in France (see below, Table 5), but not elsewhere, as far as canbe judged from the extensive collections of Sonneborn.

In this paper an account is given of the variation in the surface antigens of variety 9. In planning this work it was hoped to shed light on the following three problems: 1. the resemblances and differences between variety 9 and the two other varieties previously investigated, namely, variety 4 (Sonneborn 1950) and variety 1 (Beale 1952), concerning the antigenic system in general and its genetic determinants; 2. in particular, variation in "homologous" antigens in different varieties, and 3. the distribution in nature of the various serotypes, and their variation in successive seasons. Solution of this last problem was aided by the discovery of the occurrence of abundant material of variety 9 in a pond conveniently near to the laboratory.

Methods and Materials

The methods employed were similar to those previously described by Sonneborn (1950b). The only procedure which need be mentioned here is that concerning the sampling of the water in the field studies. Samples were taken in small tubes containing about 30 ml. of water (plus dead leaves, etc.), which were brought into the laboratory and incubated at 25° C. On the second and fourth day after collection the samples were examined, and individuals of *P. aurelia*, if found, isolated. Only one clone was isolated from any tube which contained *P. aurelia*.

Special care was necessary in the detection of heterozygotes in wild populations. After a small number of fissions, some animals from each clone were immediately tested with appropriate antisera, and if the presence of heterozygotes was suspected from the reaction of the paramecia with the antisera (as explained below, see p. 424), the animals were passed through autogamy. The ex-autogamous clones thus obtained were again tested with anti-sera, and the occurrence of two classes of homozygote recorded. It was essential to ensure that the animals did not pass through autogamy before the first serological testing. These precautions were not taken in the earlier work, and consequently the proportion of heterozygotes recorded must be regarded as a minimum one.

A list of the stocks used, together with the places and dates of collection, is given later (see Table 5, p. 424). All the material was collected specially for this work, either by the author or by Dr. G. H. Beale.

Results

a) The antigenic variation found in variety 9. Each stock of variety 9 was found to manifest more than one antigenic type, in the same manner as the stocks of varieties 4 and 1 previously investigated by Sonneborn and Beale respectively. In variety 9 two antigenic types were predominantly found: a G type at temperatures up to 28° C, and an X type at temperatures between 31° and 33° C. The G and X nomenclature is based on genetic considerations as will be explained below (p. 424).

Transformation from one type to another could be regularly produced by putting the animals at the appropriate temperature. When G types were transferred from 25° C to 31° C, transformation to the X type occurred after approximately ten fissions. The reverse transfer of X animals from 31° to 25° C brought about transformation to the G type after approximately seven fissions. All the stocks of variety 9 responded in the same way to these temperature changes.

Using the G types of five stocks (504, 505, 506, 509, 510), each derived from different wild individuals of variety 9, five different anti-sera were prepared. The homologous and heterologous reactions of these sera, as determined by the immobilization of the various types of paramecia, are shown in Table 1 (top left section). The G types of the remaining stocks of variety 9 were immobilized by one or other of these five antisera in such a way as to indicate that all stocks at present available can be classified according to one of the five G types. No marked cross reactions occurred except between types 505 G and 506 G; these two types could however be easily distinguished by their reactions with non-homologous sera, as can be seen by reference to Table 1.

Table 1. *Titration of G types in five stocks of variety 9, and their cross-reactions with seven different G types of variety 1*

Stock	Sera											
	anti-504G	anti-505G	anti-506G	anti-509G	anti-510G	anti-41G	anti-60G	anti-61G	anti-90G	anti-192G	anti-156G	anti-168G
Variety 9												
504	1/1500	—	1/100	—	—	—	—	1/50	1/1500	—	1/125	—
505	1/1000	1/1500	1/1500	1/500	1/1000	—	—	1/125	1/1500	—	—	—
506	—	1/750	1/1500	—	1/100	—	—	1/250	1/1500	—	.	.
509	1/250	—	1/250	1/1500	1/125	—	—	—	1/1500	—	—	
510	—	—	1/250	—	1/1000	—	—	1/500	1/1500	—	—	—
Variety 1												
41	—	1/500	—	1/50	1/500	1/500	—	1/250		-	—	—
60	—	—	—	—	—	—	1/500	1/50	—	—	—	.
61	—	1/500	1/200	1/50	1/750	1/200	—	1/1500	—	—	—	
90	—	1/750	—	—	1/500	—	—	—	1/1500	—	—	
192	—	—	—	—	—	—	—	—	—	1/500	—	—
156	1/50	—	—	—	—	—	—	—	—	—	1/500	1/50
168	—	—	—	—	—	—	—	—	—	—	—	1/1000

Notes. Dilutions below 1/50 not considered. The dilutions shown are those required to immobilise paramecia in 2 hours.

All variety 1 types and sera from G. H. Beale.

The X types likewise comprise a number of sub-groups. Seven kinds of antisera were prepared using the X types of stocks 503, 504, 505, 510, 530, 531 and 532. The homologous and heterologous reactions of these anti-X sera are

Table 2. *Titration of X types of seven stocks of variety 9*

Stock	Sera						
	anti-503 X	anti-504 X	anti-505 X	anti-510 X	anti-530 X	anti-531 X	anti-532 X
503	$^1/_{1000}$	$^1/_{250}$	$^1/_{125}$				
504	—	$^1/_{1500}$	—	—	—	$^1/_{250}$	$^1/_{125}$
505			$^1/_{1500}$	$^1/_{100}$			
*510 (510/1)			—	$^1/_{1000}$	$^1/_{250}$		
*530 (510/24)					$^1/_{1000}$	—	—
*531 (510/25)					—	$^1/_{750}$	$^1/_{125}$
532					—	–	$^1/_{1000}$

* For convenience of designating antigens and antisera, the stocks 510/1, 510/24 and 510/25, all from the same locality (see Table 5), are given the additional stock nos. 510, 530 and 531 respectively.

shown in Table 2. No serological cross-reactions between any of the G types and any of the X types were found at the serum concentrations used (i.e. at dilutions of $^1/_{50}$ or higher).

b) Genetical analysis of the antigenic variation. The genetic basis of the antigenic variation of variety 9 was analyzed by crossing serologically distinguishable types, by passing the hybrids thus obtained through autogamy, and by examining the segregation in the ex-autogamous F2 families. The results showed that two loci are involved. A group of antigens—designated the G types—are controlled by a series of alleles at one locus—the *g* locus, and another group of antigens—designated the X types—are controlled by another series of alleles—the *x* alleles. In this work, as in previous studies with other varieties of *P. aurelia*, antigens whose specificities are controlled by allelic genes are designated by the same letter, while antigens whose specificities are controlled by non-allelic genes are designated by a different letter. Table 3 shows the segregation data, indicating the existence of five alleles at the *g* locus, and seven alleles at the *x* locus. The *g* and *x* loci show no evidence of close linkage, as far as can be judged from the limited numbers available, as shown in Table 4.

Table 3. *Segregation of antigenic types in ex-autogamous F2 families*

Cross	25° group			31° group		
509×510	509 G	510 G	Dead	509 X	510 X	Dead
	3735	3807	c. 500	58	78	35
503×510	503 G	510 G	Dead	503 X	510 X	Dead
	69	64	12	31	44	6
504×510	504 G	510 G	Dead	504 X	510 X	Dead
	83	86	31	51	52	17
505×510	505 G	510 G	Dead	505 X	510 X	Dead
	53	62	5	51	42	7
506×510	506 G	510 G	Dead	506 X	510 X	Dead
	74	50	16	55	51	14
503×504	503 G	504 G	Dead			
	64	49	12			
530×510				530 X	510 X	Dead
				70	77	3
531×510				531 X	510 X	Dead
				60	79	11
532×510				532 X	510 X	Dead
				88	70	32
530×531				530 X	531 X	Dead
				70	59	14

It may be added that in other respects the genetic basis of antigenic variation appears to conform to the systems already described for other varieties. Thus heterozygotes containing two different antigen-determining alleles were found to develop a mixture of two antigens. No special attention has been paid to the cytoplasmic component of the system, which has been extensively studied elsewhere, but there is no reason to suppose that variety 9 differs fundamentally from varieties 1 and 4 in this respect.

Table 4. *Two factor segregation in ex-autogamous F2 from cross between stocks 509 and 510*

	Parental classes		Recombinant classes		Dead
Type expressed at 25°	509 G	510 G	509 G	510 G	
Type expressed at 31°	509 X	510 X	510 X	509 X	
	33	48	30	23	15

c) Homology of the G types of varieties 1 and 9. In variety 1, it has previously been shown (Beale 1952) that three cytoplasmically different antigenic types occur commonly: the S types at low temperatures (10–18° C), the G types at medium temperatures (18°–27°), and the D types at high temperatures (25°–35°). As stocks and sera of variety 1 were available in this laboratory it was possible to compare the antigenic types of varieties 1 and 9.

Table 5. *Survey of the G and X serotypes of variety 9*

Stock No.	Where collected	Date	G type	X type
503/1	St. Margaret's Loch, Edinburgh	Nov. 1952	503 G	503 X
503/2	St. Margaret's Loch, Edinburgh	Nov. 1952	503 G	504 X
504	Figgate's Pond, Edinburgh	Nov. 1952	503 G	504 X
505	Strathblane, near Glasgow	Nov. 1952	505 G	505 X
506	Canal, Kingsknowe, Edinburgh	Feb. 1953	506 G	504 X
509	Canal, Ratho Park, Edinburgh	Mar. 1953	509 G	504 X
518	Chantilly, France	Sept. 1953	510 G	505 X
532	Cernay-la-ville, France	Aug. 1954	510 G	532 X
533/1	Gif-sur-Yvette, France	Aug. 1954	510 G	510 X
533/2	Gif-sur-Yvette, France	Aug. 1954	510 G	504 X
534/1-6	La Fillolière (Farm pond), near Cernay, France	Aug. 1954	509 G	510 X
534/7	La Fillolière (Farm pond), near Cernay, France	Aug. 1954	509 G	503 X
534/8	La Fillolière (Farm pond), near Cernay, France	Aug. 1954	510 G	503 X
534/9	La Fillolière (Farm pond), near Cernay, France	Aug. 1954	510 G	510 X
510/1-87	Blackford Pond, Edinburgh	various	see Table 7	

The antigenic types of the middle temperature range (the G types of varieties 1 and 9), and those of the high temperature ranges (the D types of variety 1 and the X types of variety 9), were compared. Cross reactions were found between certain of the G types of the two varieties. (It was for this reason that the G types of variety 9 were designated with the letter G). No cross reactions were however found between any of the X types of variety 9 and the D types of variety 1. Finally, no cross reactions were found between any of the X types of variety 9 with any G type, whether in variety 1 or 9. In fact none of the X types cross reacted with any other known type, and they were therefore provisionally designated with the letter X.

The details of the relationships between the G types of varieties 1 and 9 are set out in Table 1. The heterologous reactions are usually (thongh not always) of a low order by comparison with the homologous reactions, and it can be seen that there is generally no one-to-one correspondence of the different sub-types of the varieties. For instance the anti-90 G serum of variety 1 immobilizes all the G sub-types of variety 9 indiscriminately, but the variety 9 anti-G sera are not reciprocally effective against type 90 G of variety 1.

All these tests were made with unabsorbed serum.

d) Distribution in nature of the various serotypes of variety 9. The majority of the antigenic types of variety 9 were found in more than a single locality, and a selection of different antigenic types could occur in the same place, as will be seen by reference to Tables 5 and 7. There is no correlation between the G antigenic type and the X antigenic type found in a particular stock. For instance serotype 503 X was originally found in stock 503 in association with antigenic type 503 G. In stocks collected later this X type—503 X—has been found in association with two other G types—509 G and 510 G.

Table 6. *Distribution of species of Paramecium in samples of water collected from Blackford Pond over a period of 15 months*

Date of collection	No. of tubes	No. of tubes containing at least one example of			
		P. aurelia variety 2	*P. aurelia* variety 9	*P. bursaria*	*P. caudatum*
18. 5. 54	31	0	0	0	17
22. 5. 54	50	1	0	0	26
26. 5. 54	50	0	1	0	26
8. 6. 54	30	0	0	0	16
2. 7. 54	25	2	1	0	20
14. 7. 54	25	0	0	0	19
17. 8. 54	24	0	0	14	13
1. 9. 54	50	1	9	20	40
21. 9. 54	29	0	12	4	18
2. 10. 54	30	1	10	0	19
16. 10. 54	50	4	21	0	30
5. 11. 54	35	0	8	2	14
24. 11. 54	45	0	11	0	14
11. 12. 54	39	0	5	2	7
5. 1. 55	50	0	2	0	10
27. 1. 55	50	0	0	1	4
9. 3. 55	42	0	0	0	1
31. 3. 55	55	0	0	0	0
20. 4. 55	54	0	0	0	0
6. 5. 55	54	0	0	4	0
26. 5. 55	50	0	0	0	0
15. 6. 55	50	0	0	0	5
7. 7. 55	50	0	0	1	15
24. 7. 55	50	0	1	10	38
7. 8. 55	50	0	0	21	36

An intensive field study of the antigenic variation in one habitat was carried out. A pond near the laboratory—Blackford Pond, Edinburgh—was sampled throughout one year (and intermittently at other times also). In this part of the work, records were made not only of *P. aurelia* variety 9, but also of *P. aurelia* variety 2 and of other species of *Paramecium* present, namely *P. caudatum* and *P. bursaria*. The pond is approximately 4000 sq. yards in area and is fed by spring water from a nearby hill. Water samples were taken from a 25 yard strip of the south-west border of the pond at intervals of about 20 days, beginning in May 1954 and continuing until August 1955. The numbers of samples containing at least one specimen of each species are shown in Table 6. *P. caudatum* predominated throughout and *P. bursaria* appeared abruptly in August 1954 and then rapidly declined. Variety 9 of *P. aurelia* appeared in high density in September during the decline of *P. bursaria*. A gradual parallel decline in numbers of *P. aurelia* and *P. caudatum* took place until at the end of March 1955 no paramecia of any species were found. Variety 2 of *P. aurelia*, although present, never appeared in any great

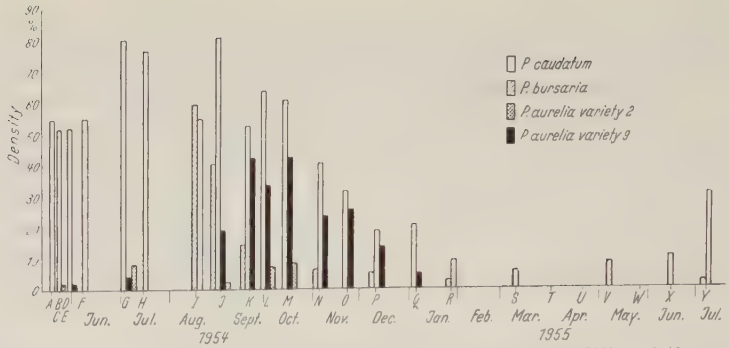
P. caudatum
P. bursaria
P. aurelia variety 2
P. aurelia variety 9
Density
A B D C E F G H I J K L M N O P Q R S T U V W X Y
Jun. Jul. Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May. Jun. Jul.
1954
1955

Fig. 1. Frequency of *Paramecium* spp. in Blackford Pond, Edinburgh, at different times of the year. The percentages given are calculated from the number of tubes containing at least one example of the given species or variety. The number of animals per tube is not considered

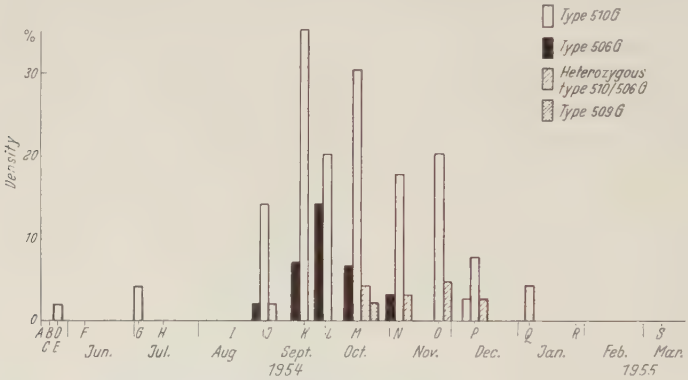
Type 510G
Type 506G
Heterozygous Type 510/506G
Type 509G
%
Density
A B D C E F G H I J K L M N O P Q R S
Jun. Jul. Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1954
1955

Fig. 2. Frequency of serotypes 510 G, 506 G, 509 G and the heterozygote 510 G/506 G of *P. aurelia*, variety 9, in Blackford Pond, Edinburgh, at different times of the year

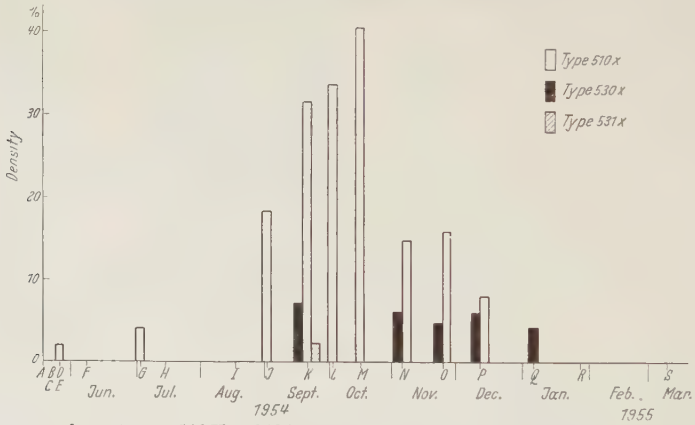

Fig. 3. Frequency of serotypes 510X, 530X and 531 X of *P. aurelia*, variety 9, in Blackford Pond. Edinburgh

numbers. Collections were made at different times during one 24 hour period. but there was no indication of any diurnal fluctuation in density. These results are expressed diagrammatically in Fig. 1.

The density of the population varied in the different years. *P. caudatum*, which occurred at high density in May 1954, was absent in May 1955 and

first appeared again in June. This difference probably resulted from the severity of the winter of 1954/55 when the pond was frozen during the whole of February and again for a time in March.

A total of eighty-seven stocks of *P. aurelia*, variety 9 were collected from Blackford Pond, and all were tested serologically. In Table 7 the serotypes of these eighty-seven stocks are set out. Three different G types were found, and three different X types. Some types occurred much more frequently than others: for example, the class of animals developing serotypes 510 G and 510 X was by far the most frequent, whilst the type 509 G was found on only a single occasion. Of special interest was the discovery of heterozygotes 506 G/510 G, which must be assumed to be the product of matings in the pond. The proportion of these heterozygotes shown in Table 7 should be regarded as a minimum estimate, as already mentioned. For technical reasons the detection of heterzygotes in nature was not attempted with the high temperature (X) types. The results of the sampling of Blackford Pond for *P. aurelia* variety 9 are shown diagrammatically in Fig. 2 for the G types and Fig. 3 for the X types. With the data at present available no obvious trends in the frequencies of the various types can be discerned.

Table 7. *Distribution of serotypes of P. aurelia, variety 9, in samples taken from Blackford Pond, Edinburgh over the period May 1954 to January 1955 (Stock 510)*

Date of collection	No. of isolations of variety 9	Serotypes	
		G type	X type
Oct. 1953	1	510 G	510 X
Jan. 1954	1	510 G	510 X
26 May 1954	1	510 G	510 X
2 July 1954	1	510 G	510 X
1 Sept. 1954	9 — 1	506 G	510 X
	— 7	510 G	510 X
	— 1	*506 G/510 G	510 X
21 Sept. 1954	12 — 2	506 G	510 X
	— 7	510 G	510 X
	— 2	510 G	530 X
	— 1	510 G	531 X
2 Oct. 1954	10 — 4	506 G	510 X
	— 6	510 G	510 X
16 Oct. 1954	21 — 3	506 G	510 X
	—15	510 G	510 X
	— 1	506 G/510 G	510 X
	— 1	506 G/510 G	undetermined †
	— 1	509 G	510 X
5 Nov. 1954	8 — 1	506 G	510 X
	— 3	510 G	510 X
	— 2	510 G	530 X
	1	510 G	undetermined †
	— 1	506 G/510 G	510 X
24 Nov. 1954	11 — 6	510 G	510 X
	— 2	510 G	530 X
	— 1	510 G	undetermined †
	— 1	506 G/510 G	510 X
	— 1	506 G/510 G	undetermined †
11 Dec. 1954	5 — 1	506 G	510 X
	— 1	510 G	510 X
	2	510 G	530 X
	— 1	506 G/510G	510 X
5 Jan. 1955	2	510 G	530 X
Aug. 1955	1	510 G	510 X
1 Sept. 1955	4	510 G	510 X
Total	87		

* Types designated 506 G/510 G are heterozygotes on collection. After passing through autogamy in the laboratory they yield the two homozygotes 506 G and 510 G.

† These were undetermined because they died before the X type could be tested for.

Apart from the main season for variety 9 (May 1954 to January 1955), sporadic collections were made both earlier and later, as shown in Table 7. However, all these odd samples produced serotypes 510 G and 510 X which as already mentioned constituted the most frequent class throughout.

Discussion

This study of the surface antigens of *P. aurelia* variety 9 shows that there is no fundamental difference between the system of genetic determinants here found, and that already analysed in varieties 1 and 4. The general situation in all the varieties is that the variation is determined by multiple alleles at several different loci, and that the expression of the genes at any particular locus is controlled by the state of the cytoplasm. However each variety has distinctive features.

For instance in variety 1 there is a very regular effect of temperature on the system of cytoplasmic states, though the exact boundaries of the temperature ranges characteristic for the different cytoplasmic states vary in the different stocks (Beale 1952, 1954). In variety 4, on the other hand, the sequence of cytoplasmic states is not so regular (Sonneborn, Ogasawara and Balbinder 1953). The present work indicates that the system in variety 9 is very similar to that of variety 1. Variety 9 differs from variety 1 however in that the temperature ranges of the two types studied (G and X) seem to be identical in all the stocks examined. In all of them the G types transform to the X type at temperatures between 28° and 31° C.

Variety 9 also resembles variety 1, and differs from variety 4, in the great serological diversity produced by the different alleles at a given locus. In variety 4 (Sonneborn 1951) the different alleles in different stocks have been found to produce only slight modifications of antigen specificity.

The similarities of varieties 1 and 9 have facilitated a systematic comparison of the antigenic types in these two varieties. The serological correspondence of the two varieties is nevertheless not very close. A few of the G types in each variety show cross reactions, indicating the possibility that the *g* loci in the two varieties show some degree of homology. However, it should be stressed that genetical homology can only be shown by genetic experiments, i.e. by making hybrids between the two varieties, and this has not been possible. In any case, none of the serotypes in variety 9 can be considered to be identical with any in variety 1.

The field study of antigenic variation of variety 9 has shown a considerable amount of polymorphism in a single habitat. Such polymorphism has been previously found in variety 1 also (Beale 1954), though the data there are less extensive than those here presented. In variety 9, as already mentioned, the Blackford Pond population has been found to contain three *g* alleles, and three *x* alleles, in various combinations. In addition, the smaller collections from other places also contained paramecia of more than one genotype. Thus, two collections from St. Margaret's Loch, Edinburgh (stock 503) yielded two *x* alleles; two collections from Gif-sur-Yvette, France (stock 533) likewise yielded two *x* alleles, and the nine collections from La Fillolière (stock 534) gave two *g* alleles and two *x* alleles, in all combinations. These facts show that serotypic polymorphism is the rule, even in small enclosed ponds.

In view of the regular occurrence of autogamy in all stocks of *P. aurelia*—a process rendering all animals completely homozygous in a single step—and in view of the isolated nature of the habitats studied, it must be inferred that there exists a selective advantage in favour of a polymorphic system. Possibly hetero-

zygotes containing two different antigen-determining alleles have an advantage over the homozygotes. The discovery of heterozygotes in the Blackford Pond population—surprising on account of the effect of autogamy—is in conformance with this view, though only experiment could make one sure of its correctness.

It is worth noting in this connection that the growth of a population of paramecia in a given habitat is extremely erratic, due to fluctuations in temperature, amount of food available, etc. In the laboratory the fission rate of *P. aurelia* variety 9 is 12 hours at 18° C, 2 weeks at 5° C (assuming excess food). The maximum water temperature of Blackford Pond was about 20° C, and in November 1954 it had dropped to 5° C. (The p_H remained constant at about 8·0 throughout the period under observation.) Hence, after passing through a period of relatively rapid growth, the population enters a static phase for most of the year. Mating may occur only once a year (or less), during the period of high density, and the heterozygotes detected in the population after this period may belong to clones which have undergone conjugation sometime previously, but still have to pass through autogamy. It is worth stressing however that the discovery of heterozygotes in nature now makes it certain that conjugation is a phenomenon to be reckoned with in wild populations, and not merely a laboratory oddity, as some workers have thought.

The results obtained so far are insufficient to indicate whether there is any change in gene frequencies from year to year, or even whether the same array of alleles is present in succeeding years. However, there seems to be no doubt that Mendelian recombination is occurring constantly in wild populations of paramecia, and is a process of fundamental importance for the structure of these populations.

Summary

1. The system of antigenic variation in *Paramecium aurelia*, variety 9, has been studied and found to be basically similar to the systems previously studied in varieties 1 and 4.

2. Two main classes of serotype have been studied in variety 9: a G type and an X type. A series of five allelic genes control the specificity of the G type antigens, and another series of seven alleles control the X types.

3. There is a regular effect of temperature on the serotypes, the G types being formed at lower temperatures (28° C or lower), and the X types at higher temperatures (31–33° C).

4. Cross reactions occur between some of the G types of variety 9 with some of the G types of variety 1. No two types in different varieties are however identical. No cross reactions have been observed between the X types of variety 9 and the D types of variety 1.

5. The distribution in nature of members of the two sets of alleles, controlling the G and X antigens respectively, has been studied in detail. Eighty-seven samples of *P. aurelia* variety 9 were collected from one pond in Edinburgh. Considerable serological polymorphism was found, and six collections were shown to be heterozygotes bearing two *g* alleles. The occurrence of these heterozygotes proves for the first time that conjugation of paramecia occurs in nature.

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Aus dem Institut für Entwicklungsphysiologie an der Universität Köln

ZUR FRAGE NACH DEM EINFLUSS DER TEMPERATUR BEI DER AUSLÖSUNG VON CHROMOSOMENMUTATIONEN IN DER MEIOSIS DURCH RÖNTGENSTRAHLEN

Von

LUISE STANGE

(Eingegangen am 31. Dezember 1955)

Einleitung

In Untersuchungen über den Einfluß von Begleitfaktoren auf die Mutationsauslösung durch Röntgenstrahlen in der Meiosis von *Oenothera* hatte sich die Temperatur als ein das Mutationsgeschehen beeinflussender Faktor erwiesen (STANGE 1951). Während der Prozentsatz der manifest werdenden Chromosomenbrüche in Versuchen bei 10 und 20° C gleich blieb, war die relative Häufigkeit der Translokationen bei 20° C gegenüber der bei 10° C vermindert, die relative Häufigkeit der Fragmente dagegen deutlich erhöht. Im Gegensatz zu Untersuchungen anderer Autoren über die Temperaturwirkung bei der Mutationsauslösung waren in den Versuchen an *Oenothera* die verschiedenen Temperaturen über die ganze Versuchsdauer beibehalten worden. Dabei ist es selbstverständlich nicht möglich, Aussagen über die der Temperatur gegenüber empfindlichen Stadien bzw. Prozesse zu machen. Es ergab sich die Forderung, an dem gleichen Objekt und unter sonst gleichartigen Bedingungen den Zeitpunkt und die Dauer der Einwirkung der verschiedenen Temperaturen zu variieren. Die in diesem Sinne geplanten Versuche konnten allerdings bisher nur unvollständig durchgeführt werden. Da jedoch im Augenblick die Möglichkeit für eine Fortsetzung der Versuche nicht besteht, sollen die bereits vorliegenden Ergebnisse mitgeteilt werden.

Material und Methode

Die Versuche wurden im Juli 1950 und Juli 1951 im Botanischen Institut Freiburg i. Br. durchgeführt. Für die Bereitstellung des Pflanzenmaterials und der notwendigen Institutseinrichtungen sowie für sein Interesse an den Versuchen sei Herrn Professor Dr. F. OEHLKERS auch an dieser Stelle gedankt.

Als Versuchspflanze wurde wie in den älteren Untersuchungen (STANGE 1951) der Bastard *Oenothera (suaveolens sulfurea* × *Hookeri) flavens* · ^{h}*Hookeri* verwendet. Die Vorbehandlung und Aufstellung der Pflanzen, die Fixierung, Färbung und cytologische Auswertung erfolgte in der 1951 ausführlich beschriebenen Weise.

Für die Bestrahlungen wurde ein Diax-Röntgenapparat Koch und Sterzel mit Multix-Röhre MWG Do 45/220 mit eingebautem Hammerdosimeter verwendet, 31,7 r/min, HWS 1,67 Al. In allen Versuchen betrug die Dosis 300 r.

Im Jahre 1950 wurden folgende Versuchsgruppen durchgeführt:

1. Die verschiedenen Temperaturen wurden nur *während* der Bestrahlung (am 1. Versuchstag um 11 Uhr) gegeben. Vor- und hinterher standen die Pflanzen in den temperaturkonstanten Kammern bei 15° C. Da in dem Raum, in dem der Röntgenapparat stand, keine Temperaturregulation möglich war, wurden die Sprosse zur Bestrahlung in Standzylinder mit Wasser der entsprechenden Temperatur so gesteckt, daß die Sproßgipfel mit den zu bestrahlenden Blütenknospen gerade noch mit Wasser bedeckt waren. Während der etwa 10 min, in denen die Pflanzen außerhalb des temperaturkonstanten Raumes waren, veränderte sich

die Temperatur des Wassers nicht meßbar. Die Fehler in der Dosierung, die durch Absorption durch die Wasserschicht bedingt waren, dürften unbedeutend und für alle Temperaturen praktisch gleich groß gewesen sein.

a) 5° C, b) 10° C, c) 20° C, d) 25° C.

2. Die verschiedenen Temperaturen wurden *5 Std* lang direkt *vor* der Bestrahlung gegeben, vor- und hinterher 15° C. Die Bestrahlung erfolgte am 1. Versuchstag um 17 Uhr.

a) 10° C, b) 20° C.

3. Die verschiedenen Temperaturen wurden *5 Std* lang direkt *nach* der Bestrahlung gegeben, vor- und hinterher 15° C. Die Bestrahlung erfolgte am 1. Versuchstag um 12 Uhr.

a) 10° C, b) 20° C.

4. Die Temperaturen blieben über die ganze Versuchsdauer *konstant*. Bestrahlungen am 1. Versuchstag um 18 Uhr.

a) 10° C, b) 15° C, c) 20° C.

Die Knospen aller Versuche wurden am 5. Tag nach Versuchsbeginn vormittags fixiert, mit Ausnahme des Versuches mit konstanter Temperatur von 20° C, bei dem wegen des beschleunigten Ablaufes der Meiosis schon am 4. Tage nach Versuchsbeginn fixiert wurde.

Im Jahre 1951 wurden auf Grund der Ergebnisse des Jahres 1950 folgende Versuche durchgeführt:

1. Die verschiedenen Temperaturen wurden *12 Std* lang direkt *nach* der Bestrahlung gegeben, sonst konstant 15° C. Bestrahlungen am 1. Versuchstag zwischen 9 und 10 Uhr.

a) 10° C, b) 20° C. Fixierung in beiden Fällen am 5. Tag.

2. Die verschiedenen Temperaturen wurden *36 Std* lang direkt *nach* der Bestrahlung gegeben, sonst konstant 15° C. Bestrahlungen am 1. Versuchstage zwischen 9 und 10 Uhr.

a) 10° C, b) 20° C. Fixierung in beiden Fällen am 5. Tag.

3. Die Temperaturen blieben über die ganze Versuchsdauer *konstant*.

a) 10° C, Fixierung am 5. und 6. Tag; b) 15° C, Fixierung am 5. Tag; c) 20° C, Fixierung am 4. und 5. Tag.

4. Bei konstanter Temperatur von 15° C während der ganzen Versuchsdauer wurde die Bestrahlungsdosis von 300 r über verschieden lange Zeiträume *fraktioniert* gegeben, jeweils in 6 Einzeldosen von 50 r.

a) 300 r über 1 Std verteilt, b) über 5 Std verteilt, c) über 12 Std verteilt, d) über 36 Std verteilt. Fixierung in allen Fällen am 5. Tag.

Zu allen Versuchen liefen unbestrahlte Kontrollen mit der entsprechenden Temperaturbehandlung (in den Tabellen mit großen Buchstaben bezeichnet).

Versuchsergebnisse

Die Versuchsergebnisse des Jahres 1950 sind in Tabelle 1 zusammengestellt. Im oberen Teil der Tabelle finden sich die unbestrahlten Kontrollen mit den verschiedenen Temperaturbehandlungen. Obgleich 2 Kontrollversuche nicht ausgewertet wurden und sich in der Kontrolle bei konstant 20° C keine analysierbaren Zellen finden ließen, geht doch aus den Ergebnissen hervor, daß die Temperaturbehandlungen allein keinen Einfluß auf die spontane Mutationsrate ausübten.

In der Kontrolle bei konstanter Temperatur von 20° C wurde trotz gründlicher Durchsicht des gesamten fixierten Knospenmaterials keine einzige analysierbare Diakinese gefunden. Schon beim Fixieren war dieser Versuch durch seine welken Knospen aufgefallen. Bei der Anfertigung der cytologischen Präparate ließen sich die Antheren zum großen Teil nicht quetschen. In ihren Loculi lagen völlig degenerierte Zellen in dunklen Strängen zusammengeballt. Sie glichen damit den 20°-Versuchen des Jahres 1949 (STANGE 1951), die nach 4 Tagen fixiert worden waren, während sich damals in den nach 3 Tagen fixierten Versuchen gut analysierbare Diakinesen in großer Anzahl finden ließen.

So wie die Kontrolle zeigte auch der Bestrahlungsversuch bei konstant 20° C (4c) schwere Störungen. Es konnten im ganzen nur 10 Diakinesen analysiert

werden, von denen 8 jeweils 1 bis mehrere fragmentierte Chromosomen aufwiesen. In den beiden anderen Versuchen bei konstanter Temperatur (4a und b) finden sich die gleichen Verhältnisse wie in den Versuchen 1949 wieder: Bei etwa gleicher Anzahl von Chromosomenbrüchen bei den beiden Temperaturen ist der Anteil der Fragmente bei 15° C statistisch gesichert größer als bei der Temperatur von 10° C und der Anteil der Translokationen kleiner. Dabei betrug bei diesen Versuchen die Differenz zwischen den Temperaturen nur 5° C.

In der 1. Versuchsgruppe wirkten die verschiedenen Temperaturen nur während der Bestrahlung auf die Pflanzen ein. Leider ist in den Ergebnissen die Voraussetzung für eine varianzanalytische Bearbeitung nicht gegeben. Bei der innerhalb der einzelnen Versuche vorhandenen Variabilität kann im χ^2-Test erst ein P-Wert von $< 0{,}01$ als gesichert angesehen werden. Wie die Ergebnisse in der Tabelle 1 zeigen, läßt sich kein gesicherter Unterschied zwischen den verschiedenen Temperaturen nachweisen. Das betrifft

Tabelle 1. *Versuche 1950*

Versuch	Versuchsdauer Tage	Analysierte Chromosomen	Gebrochene Chromosomen			Translocierte Chromosomen				Fragmentierte Chromosomen			
			%	χ^2	P		%	χ^2	P		%	χ^2	P
Unbestrahlte Kontrollen													
1A) 10 min 5°; 15°	4	4130	11	0,266		10	0,242			1	0,024		
1B) 10 min 10°; 15°	4	2842	12	0,422		12	0,422			—	—		
1C) 10 min 20°; 15°	4	2800	4	0,143		4	0,143			—	—		
2/3A) 5 Std 10°; 15°	4	2800	9	0,321	6,61	8	0,286			1	0,036		
2/3B) 5 Std 20°; 15°	4	2800	4	0,143	> 0,20	4	0,143			—	—		
4B) konstant 15°	4	2800	6	0,214		6	0,214			—	—		
4C) konstant 20°	3	—	—	—		—	—			—	—		
Bestrahlung 300 r													
1a) während Bestrahlung 5°; 15°	4	1526	19	1,245		6	0,393			13	0,852		
1b) während Bestrahlung 10°; 15°	4	4200	70	1,667	9,42	38	0,905	9,94	> 0,01	32	0,762	3,26	> 0,30
1c) während Bestrahlung 20°; 15°	4	4200	44	1,048	> 0,02	22	0,524			22	0,524		
1d) während Bestrahlung 25°; 15°	4	1400	11	0,786		4	0,286			7	0,500		
2a) 5 Std vor Bestrahlung 10°; 15°	4	4270	46	1,077		16	0,375			30	0,703		
2b) 5 Std vor Bestrahlung 20°; 15°	4	5600	51	0,911	2,41	26	0,464	2,92	> 0,30	25	0,446	4,78	> 0,10
3a) 5 Std nach Bestrahlung 10°; 15°	4	4200	50	1,190	> 0,30	18	0,429			32	0,762		
3b) 5 Std nach Bestrahlung 20°; 15°	4	4214	50	1,187		26	0,617			24	0,570		
4a) konstant 10°	4	2004	31	1,547	0,19	30	1,497	1,96	> 0,10	1	0,050	10,80	< 0,01
4b) konstant 15°	4	2870	49	1,707	> 0,50	30	1,045			19	0,662		
4c) konstant 20°	3	140	15	10,714		—	—			15	10,714		

Tabelle 2. *Versuche 1951*

Versuch	Versuchsdauer Tage	Analysierte Chromosomen	Gebrochene Chromosomen			Translocierte Chromosomen			Fragmentierte Chromosomen		
			%	χ^2	P	%	χ^2	P	%	χ^2	P
Unbestrahlte Kontrollen											
1A) 12 Std 10°; 15°	4	2800	5 0,179			2 0,071			3 0,107		
1B) 12 Std 20°; 15°	4	2800	7 0,250			4 0,143			3 0,107		
Bestrahlung 300 r											
1a) 12 Std nach Bestrahlung 10°; 15°	4	3864	67 1,734	0,31	> 0,50	20 0,518	1,10	> 0,20	47 1,216	0,003	> 0,90
1b) 12 Std nach Bestrahlung 20°; 15°	4	3080	59 1,916			22 0,714			37 1,201		
2b) 36 Std nach Bestrahlung 20°; 15°	4	630[1]	98 15,556	10,55	< 0,01	2 0,318	0,49	> 0,30	96 15,238	11,94	< 0,001
			Versuch 3c			Versuch 3c			Versuch 3c		
3a) konstant 10°	4	1204	21 1,744	0,10	> 0,70	12 0,997	1,41	> 0,20	9 0,748	1,93	> 0,10
			Versuch 3b			Versuch 3b			Versuch 3b		
3a) konstant 10°	5	210	2 0,952			2 0,952			— —		
3b) konstant 15°	4	2800	53 1,893			18 0,643			35 1,250		
3c) konstant 20°	3	1070	106 9,907			6 0,561			100 9,346		
3c) konstant 20°	4	—	— —			— —			— —		
4a) 15° 6×50 r in 1 Std	4	2562	54 2,108	2,75	> 0,05	18 0,703	1,16	> 0,20	36 1,405	1,61	> 0,20
4c) 15° 6×50 r in 12 Std	4	2919	44 1,507			14 0,480			30 1,028		

[1] Weitere 300 Zellen mit gleichartigen Schädigungen, nicht bis ins einzelne analysiert.

sowohl die Anzahl der manifest gewordenen Brüche als auch das Verhältnis zwischen den verschiedenen Aberrationstypen, Translokationen und Fragmenten. Es muß daraus geschlossen werden, daß die Temperatur während der Bestrahlung keinen Einfluß auf das Mutationsgeschehen hatte.

Das gleiche gilt für die 2. und 3. Versuchsgruppe. Bei einer ausreichenden Anzahl analysierter Zellen (vgl. Analyse der Streuung 1951) stimmen sowohl die Anzahlen der aufgefundenen Brüche als auch die Anteile an Translokationen und Fragmenten in den einzelnen Versuchen gut überein. Eine Temperatureinwirkung von 5 Std Dauer reicht also nicht aus, um das Ergebnis der Mutationsauslösung durch Röntgenbestrahlung zu beeinflussen, unabhängig davon, ob sie unmittelbar vor oder nach der Bestrahlung gegeben wird.

Auf Grund dieser Befunde wurden die Pflanzen bei der Fortsetzung der Versuche im Sommer 1951 nach der Bestrahlung längere Zeiten den verschiedenen Temperaturen ausgesetzt. Die in Tabelle 2 dargestellten Ergebnisse zeigen, daß auch eine Einwirkung der verschiedenen Temperaturen von 12 Std Dauer (1a und b) keine Differenzen in der Zahl und Natur der auftretenden Aberrationen ergibt. Dagegen bietet der Versuch mit 36 Std 20° C (2b) ein völlig verändertes Bild.

Mit einer Häufigkeit von 15,5% traten Chromosomenbrüche in einem Ausmaße auf, wie sie größenordnungsmäßig nur noch in dem Versuch mit konstanter Temperatur von 20° C (3c) beobachtet wurden. Dieser Erhöhung der Bruchanzahl liegen ausschließlich Fragmentationen zugrunde.

Vergleicht man die Versuche bei konstanten Temperaturen (3a—c), so findet man eine Differenz gegenüber den Versuchsergebnissen des Jahres 1949: Die Anzahl der aufgefundenen Chromosomenbrüche ist im 20°-Versuch im Vergleich mit den beiden anderen Temperaturen stark erhöht. Diese Erhöhung kommt allein durch eine Vermehrung der Fragmente zustande. Im 10°- und 15°-Versuch ist die Zahl der Chromosomenbrüche etwa gleich groß, während der Anteil der Fragmente an den Aberrationen im 15°-Versuch wiederum — diesmal allerdings nicht statistisch gesichert — höher liegt als bei der Temperatur von 10° C. Ganz allgemein liegt in diesem Versuchsjahr der Prozentsatz der Fragmente in sämtlichen Versuchen höher als in den Vorjahren. Wodurch diese Unterschiede in den Ergebnissen der verschiedenen Versuchsjahre begründet sind, muß ungeklärt bleiben. — Der nach 4 Tagen fixierte 20°-Versuch zeigte die bereits bekannten Störungen; es wurden keine analysierbaren Diakinesen gefunden.

Von den Versuchen mit Fraktionierung der Röntgendosis wurden nur diejenigen ausgewertet, bei denen die Bestrahlung über 1 Std und über 12 Std verteilt war (4a und c). Die Ergebnisse beider Versuche stimmen sowohl in der Anzahl der aufgefundenen Chromosomenbrüche als auch in bezug auf die Häufigkeit der beiden Aberrationstypen untereinander und mit dem Versuch 3b, 300 r in 9,46 min, gut überein.

Besprechung der Ergebnisse

In den Versuchen des Jahres 1950 zeigte sich, daß eine Einwirkung verschiedener Temperaturen weder zur Zeit der Bestrahlung noch 5 Std lang unmittelbar vor oder nach der Bestrahlung eine Veränderung des röntgeninduzierten Mutationsgeschehens in der Meiosis von *Oenothera* hervorrief. Bei Verlängerung der Einwirkungszeit nach der Bestrahlung im Jahre 1951 auf 12 bzw. 36 Std ergaben sich auch bei 12stündiger Einwirkung keine Differenzen zwischen 10 und 20° C. Die Ergebnisse entsprechen denjenigen für konstante Temperatur von 15° C. Erst bei einer Einwirkungsdauer von 36 Std ergab sich für die Temperatur von 20° C eine starke Vermehrung der Anzahl von Fragmenten. Dieser Versuch glich damit den Versuchen bei konstanter Temperatur von 20° C. Die unbestrahlte Kontrolle bei konstant 20° C zeigte keine Erhöhung der spontanen Mutationsrate.

Die Deutung dieser Versuche wird dadurch erschwert, daß die Temperatur unabhängig vom Mutationsgeschehen einen Einfluß auf die Geschwindigkeit des Zellteilungsablaufes ausübt, der nur schwer zu erfassen ist. Orientierende Untersuchungen (1951, S. 500) über die Geschwindigkeit des Ablaufes der Meiosis von *Oenothera* bei den verschiedenen Versuchstemperaturen hatten ergeben, daß bei 10° C die nach 4 Tagen fixierten Diakinesezellen sich während der Bestrahlung in der frühen Prophase, wohl im Leptotän, befanden, während bei 20° C die nach 4 Tagen fixierten Diakinesen zur Zeit der Bestrahlung noch prämeiotische Ruhekerne waren. Diese Befunde stimmen grundsätzlich mit den Ergebnissen ausführlicher Untersuchungen von Linnert (1951) über die Dauer der meiotischen Stadien im Freiland und bei konstant 10° C überein. Es wurde angenommen,

daß bei 20° C bei einer Versuchsdauer von 3 Tagen etwa die gleichen prämeiotischen Stadien von der Bestrahlung getroffen wurden wie in dem 10°-Versuch bei einer Versuchsdauer von 5 Tagen. Zumindest geht aus diesen Bestimmungen hervor, daß der Ablauf der Meiosis bei höherer Temperatur beschleunigt und nicht etwa durch sekundäre Störungen verzögert war. Da mit einer verschiedenen Empfindlichkeit der frühen meiotischen Prophase und der prämeiotischen Ruhekerne in verschiedenem zeitlichem Abstand vor dem Einsetzen der Meiosis gegenüber der Bestrahlung gerechnet werden muß, ist nicht mit Sicherheit auszuschließen, ob nicht eine solche unterschiedliche Empfindlichkeit die Ursache für die Differenzen bei den verschiedenen Temperaturbehandlungen darstellt. Gegen diese Annahme sprechen allerdings die Befunde des Jahres 1949. Es wurden damals keine Unterschiede zwischen den Ergebnissen der nach 4 und 5 Tagen fixierten 10°-Versuche bzw. der nach 3 und 4 Tagen fixierten 20°-Versuche gefunden, obgleich in diesen Versuchen die Bestrahlung mit Sicherheit verschiedene Stadien des Zellgeschehens getroffen hatte. Wahrscheinlicher ist daher die Annahme, daß die Temperaturwirkung über eine Beeinflussung der auf die Bestrahlung folgenden Prozesse zustande kommt. Diese temperaturempfindlichen Prozesse würden bei *Oenothera* 12—36 Std nach der Bestrahlung ablaufen oder erst bei einer Einwirkungsdauer der Temperaturen von mehr als 12 Std beeinflußbar sein.

Über die Dauer der auf eine Bestrahlung folgenden Restitutions- und Rekombinationsvorgänge wurde vor allem auf Grund von Versuchen mit protrahierter Bestrahlung an der Pollenmitose von *Tradescantia* diskutiert. Während Sax (1941), Sax und Luippold (1952) und de Serres und Giles (1953) auf der einen Seite bei fortschreitender Protrahierung bis zu 12 Std bereits von 1 Std an eine Abnahme der 2-Bruch-Ereignisse finden und aus diesen Versuchen schließen, daß die meisten Chromosomenbrüche innerhalb weniger Minuten nach der Bestrahlung wieder verheilen, beobachtete Lane (1951, 1952) auf der anderen Seite bei zunehmender Protrahierung nach einem anfänglichen Abfall einen erneuten Anstieg in der Anzahl der Chromosomenaberrationen. Er nimmt eine unterschiedliche Empfindlichkeit der auf die erste Bestrahlung folgenden Prozesse gegenüber weiteren Bestrahlungen an, die vermutlich auf eine Beeinflussung des Nukleinsäure-Stoffwechsels zurückzuführen ist. Er verwirft die Theorie der schnellen Wiedervereinigung und gibt für sie einen Zeitraum von 8 Std nach der Bruchentstehung an.

Nach 30 min langer und über 23 Std protrahierter Bestrahlung der Ruhekerne von Spermatocyten I der Heuschrecke *Gesonula punctifrons* fanden Ray-Chaudhuri und Pyne (1954) keine Unterschiede in der Häufigkeit der Anaphase-I-Brücken. Es ist möglich, daß in der Meiosis gerade in bezug auf das Restitutionsverhalten andere Verhältnisse vorliegen als in der Mitose. Auch in der Meiosis von *Oenothera* ergaben sich bei Protrahierung der Bestrahlung keine Unterschiede für Zeitspannen von 9,46 min, 1 Std und 12 Std. Dieser Befund stimmt gut überein mit der aus den Temperaturversuchen abgeleiteten Annahme, daß für die Entstehung der Chromosomenmutationen maßgebende Prozesse in einem Abstand von 12—36 Std nach der Bestrahlung ablaufen.

Auf Grund der Ergebnisse von Versuchen, in denen die Röntgenbestrahlung mit einer anschließenden Infrarot-Bestrahlung kombiniert war, wurden weitere

Schlüsse auf die auf die Bestrahlung folgenden Prozesse gezogen. Kaufmann, Hollaender und Gay (1946) und Kaufmann und Wilson (1949) nehmen an, daß die Infrarot-Behandlung die Rekombination der gebrochenen Chromosomen fördert auf Kosten der Restitution. Als Erklärung für das verschiedene Bruchverhalten werden Unterschiede im Zeitpunkt dieser beiden Verheilungsweisen angenommen. Swanson und Hollaender (1946) und Yost (1951, 1952) folgern aus ihren Kombinationsversuchen, vor allem nach protrahierter Infrarot-Bestrahlung, daß ein durch die Röntgenbestrahlung zunächst nur aktivierter Zustand am Chromosom durch die Infrarot-Bestrahlung manifest wird.

In den vorliegenden Untersuchungen an der Meiosis von *Oenothera* spricht die Erhöhung der Gesamtanzahl von Chromosomenbrüchen für eine Förderung der zum manifest werdenden Bruch führenden Prozesse durch erhöhte Temperatur, sei es über intermediäre Stoffwechselprozesse in der Zelle oder direkt am betroffenen Chromosomenort. Andererseits könnte unter Berücksichtigung der Tatsache, daß nur die Anzahl der Fragmentationen erhöht, die der Translokationen dagegen vermindert ist, auch angenommen werden, daß durch die höhere Temperatur im Anschluß an die Bestrahlung ganz allgemein die Wiederverheilungsvorgänge, sowohl Restitution als auch Rekombination, erschwert werden. In jedem Falle wäre der Zeitraum für das Wirksamwerden der vorangegangenen Bestrahlung oder für eine Beeinflussung der Rekombinations- und Restitutionsvorgänge auf 12—36 Std nach der Bestrahlung festzulegen.

Zusammenfassung der Ergebnisse

1. Infloreszenzen von *Oenothera (suaveolens sulfurea* × *Hookeri) flavens* · ^{h}*Hookeri* wurden mit 300 r bestrahlt und in verschiedener zeitlicher Beziehung zur Bestrahlung verschiedenen Temperaturen ausgesetzt, bei einer sonst in allen Versuchen gleichen Temperatur von konstant 15° C. Die Chromosomenschäden wurden in der direkt auf die Bestrahlung folgenden Meiosis der Pollenmutterzellen analysiert.

2. Temperaturen von 5, 10, 20 und 25° C, nur während der Bestrahlung geboten, hatten keinen Einfluß auf die Anzahl der induzierten Chromosomenbrüche und das Verhältnis von Translokationen zu Fragmentationen.

3. Das gleiche gilt für Versuche, in denen die Pflanzen 5 Std vor oder nach der Bestrahlung und 12 Std nach der Bestrahlung bei 10 und 20° C aufgestellt waren.

4. Dagegen war die Anzahl von Chromosomenbrüchen bei 36stündiger Behandlung mit 20° C nach der Bestrahlung und bei konstanter Temperatur von 20° C gegenüber den Versuchen bei niedrigerer Temperatur stark erhöht. Diese Erhöhung kommt ausschließlich auf Kosten der Fragmentationen zustande; die Anzahl der Translokationen war dabei stets herabgesetzt. In der gleichen Richtung liegen bereits die Differenzen zwischen den Versuchen bei konstant 15° C und konstant 10° C.

5. Unter der Annahme einer gleichen Empfindlichkeit der von der Bestrahlung getroffenen Zellen in den verschiedenen Versuchen läßt sich die Temperaturwirkung mit einer Beeinflussung der auf die Bestrahlung folgenden Reaktionen erklären, die sich bis über 12—36 Std nach der Bestrahlung erstrecken müssen.

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CHROMOSOME NUMBER AND MORPHOLOGY OF *DROSOPHILA SILVESTRIS* BASDEN — WITH A DESCRIPTIVE NOTE ON THE INTERNAL GENITALIA OF THE ADULTS

By

G. R. Knight

With 3 figures in the text

(Eingegangen am 12. November 1955)

The external characters of *Drosophila silvestris*, a member of the *obscura* group, was described by Basden (1954). The object of the present paper is to record the chromosome number and structure of *D. silvestris*, and to give a brief description of the internal genitalia of the adult, as well as the egg and puparium.

Material and Methods

The material used for this investigation came from Lugton, Midlothian, Scotland, (Stock 351). Squash preparations of more than one hundred larval brains were made, and from the large ganglion cells the metaphase chromosome configuration and morphology were studied. Larval brains were dissected in 45% aceto-carmine, allowed to remain in the stain-fixative for 5 to 10 min, then transferred to an albumenised slide for squashing. The method of squashing was as follows. The material was covered with a strip of thin cellophane. On top of the cellophane were placed two sheets of filter paper, and immediately, a large diameter specimen tube was rapidly rolled over the whole to make a one-celled layer preparation. The proper pressure required to make an evenly spread film was obtained by trial. Directly after squashing, the prepared slide is transferred to a jar of water, where the cellophane strip falls off, in about 30 sec. The slide was then transferred to 70% alcohol. Examination of the aceto-carmine preparation can be done at this stage, or, if required, the squash can be permanently stained by the Feulgen technique. All the preparations used in this study were stained by Feulgen.

Results

Examination of the squash preparations showed that the chromosome number of the diploid cell is 12, ($n = 6$), five pairs of autosomes and heteromorphic X and Y. The morphology of the autosomes is:—2 pairs of rods; 2 pairs of V's; 1 pair of dots. In the female XX are two V-shaped elements, while the male has a V-shaped X and a J-shaped Y, (Fig. 1). Structural details of the individual elements were studied, and a numerical index was assigned to each chromosome.

Chromosome-X is in the form of a V. It is the shortest of the three V-shaped chromosomes. In some of the cells, one arm appeared to be a little longer than the other, while in others, the difference in length of each arm was not obvious. The centromere was not clearly seen.

Chromosome-Y is in the form of a J. Its whole length is a little longer than that of the two arms of X: The position of the centromere has not been determined. In some cells the bottom of the J appeared bent only at a slight angle. This part of the Y chromosome was always darkly stained (Feulgen technique):

The same applied to the distal end of one arm of X, the longer arm when a difference in length between the arms was noticed.

Chromosome-2 is in the form of a V. It is slightly larger than the X-chromosome with one arm longer than the other. No secondary constriction has been seen in either of the arms of this chromosome.

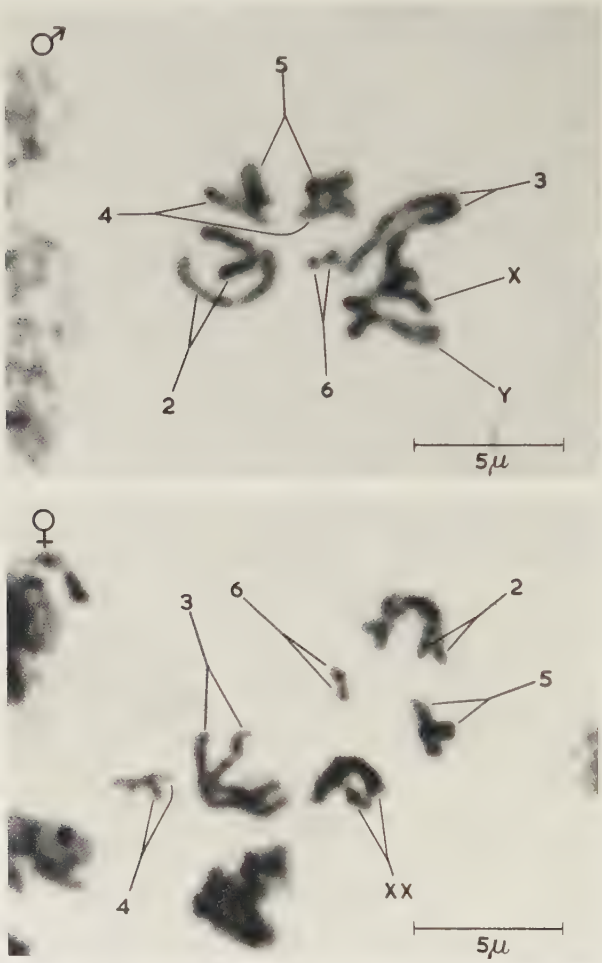

Fig. 1. Metaphase Chromosome Configuration of *D. silvestris*. Photo micrographs of large ganglion cells of ♂ and ♀ larval brain

Chromosome-3 is in the form of a V. It is the largest chromosome. The shorter arm is about equal in length to the smaller arm of chromosome-2. In the longer arm, which is fully one and a quarter times the shorter, there is a secondary constriction about one third from the distal end, so that in consequence the longer arm often appears bent.

Chromosome-4 is in the form of a rod. It is about the length of the long arm of *chromosome-2*.

Chromosome-5 is in the form of a rod. It is shorter and broader than chromosome-4, and almost always appears densely stained.

Chromosome-6 is in the form of a dot. It is clearly visible in all wellspread metaphase mitotic cells. Occasionally the dot appears slightly elongated.

Fig. 2 shows in semidiagrammatic form male and female metaphase mitotic figures selected from many drawings made from squash preparations.

Amongst other species in the *obscura* group, the most similar in metaphase chromosome configuration to *D. silvestris* is *Drosophila obscuroides* Pomini (Buzzati-Traverso, 1942). The main difference is in the shape of the Y element, which is rod-shaped in *D. obscuroides*, whereas in *D. silvestris* it is in the form of a J.

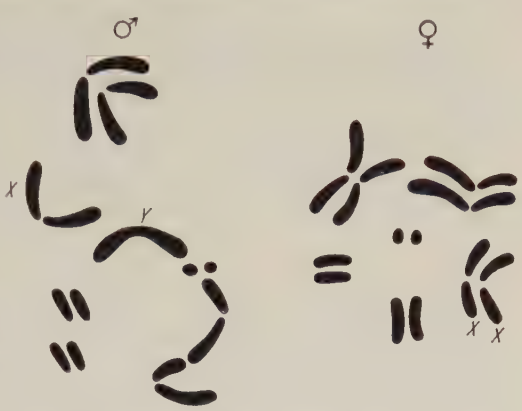

Fig. 2. Semi-diagrammatic representation of mitotic chromosomes of *D. silvestris*

Internal genitalia, egg, and puparium

Ventral Receptacle (*VR.*) (Fig. 3a). Small S-shaped tube lying against ventral surface of the uterus. Proximal two fifths of the tube is wide, the remaining distal three fifths gradually tapering.

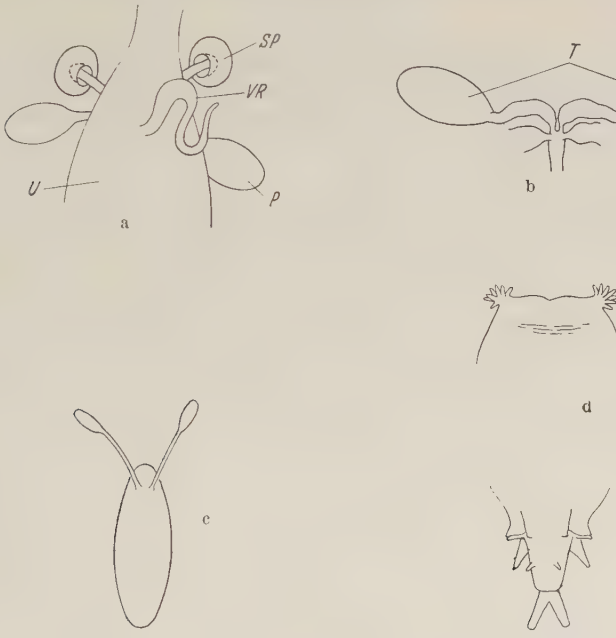

Fig. 3 a—d. *D. silvestris* — a and b, internal genitalia of the adult. (*U* uterus, *SP* spermathecae, *VR* ventral receptacle, *P* parovaria, *T* testes). c. Egg d. Anterior and posterior ends of the puparium

Spermathecae (*SP.*) (Fig. 3a). Small spherical bodies, dark brown in colour and chitinized.

Parovaria (*P.*) (Fig. 3a). Oval-shaped bodies, posterior to and extending slightly beyond the spermathecae.

Testes (*T.*) (Fig. 3b). Bright, opaque, orange-coloured, sac-like in appearance, without coiling.

Other characters

Eggs (Fig. 3c). Two filaments, attached about one fifth length of egg from apex, thin, distal ends spatulate. Length of filament about three quarters length of egg.

Puparia (Fig. 3d). Pale brownish yellow. Each of the two anterior spiracles has seven branches without definite stalk. Diagram shows ventral view of the anterior and posterior ends of the puparium.

Summary

The metaphase chromosome set of *Drosophila silvestris* Basden, a species of the *obscura* group, is described. From preparations of larval brains it was found that the diploid cell contains twelve chromosomes — five pairs of autosomes (2 V's, 2 rods, 1 dot) and heteromorphic X and Y (V- and J-shaped respectively).

Some internal characters of the adult have also been described.

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STUDIES ON FEMALE-STERILITY GENES IN *DROSOPHILA MELANOGASTER*

I. THE EFFECTS OF THE GENE *DEEP ORANGE* ON EMBRYONIC DEVELOPMENT*

By

S. J. Counce

With 10 figures in the text

(Eingegangen am 11. November 1955)

Introduction

The major groups of the animal kingdom differ profoundly from one another not only in adult morphology, but often in the fundamental structure of the eggs from which the adults arise. Several authors (e. g. Waddington 1940, 1951; Dalcq 1951) have emphasised the importance for macro-evolutionary processes of genes which alter the egg structure. In general such genes are difficult to detect, and comparatively little is known about them. One particular class of them, however, may be discovered more readily; namely those which, in females, so affect the maturation of the eggs that they cannot develop into adults, the female remaining sterile.

Some interesting studies have already been made of mutants in *Drosophila* which cause female-sterility. Lynch (1919) made a careful genetic study of several such genes. Later physiological and morphological studies, especially those of Clancy and Beadle (1937), Anderson (1945) and Beatty (1949), have shown that sterility can be caused by either physiological or mechanical defects. Beatty showed further that development of the egg cell or zygote could cease at any one of several stages, from failure of oöcyte formation to the absence of the F_1 in crosses involving males of certain genotypes.

Lynch (1919) was the first to describe mutants of the latter type. When females homozygous for the sex-linked gene *fused* (*fu*) are mated to *fu* males, although eggs are produced, all zygotes die during the course of embryonic development (Fig. 1a). If the father is non-*fu*, the heterozygous daughters are viable but the male zygotes die as embryos (Fig. 1b). The *fu* male is perfectly fertile with all other females, and if mated to a heterozygous female, viable offspring are produced in the expected Mendelian ratio (Fig. 1c). Lynch also found that the sex-linked gene *rudimentary* (*r*) varied only in degree from *fu*, in that sons were produced rarely in matings of non-mutant males to mutant females. Merrell (1947) found that the sex-linked mutant *deep orange* (*dor*) also exhibited this unusual type of female-sterility. All three genes cause death during the embryonic stage. This type of sterility is not entirely limited to sex-linked genes (Lynch, 1919; Redfield, 1926) and if the gene is autosomal, its lethal effect can be counteracted by the presence of its normal counterpart

* This paper is taken from a thesis presented to the Senatus of the University of Edinburgh in partial requirement for the degree Doctor of Philosophy.

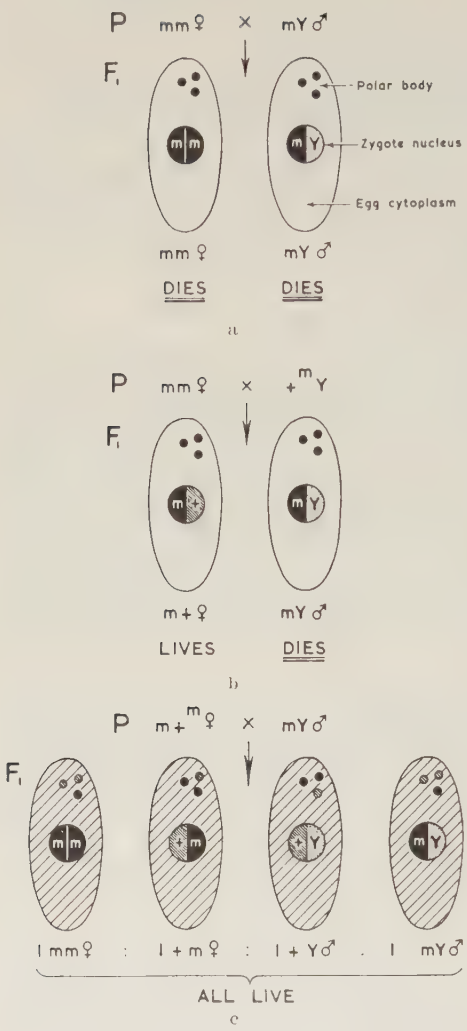

Fig. 1a—c. Schematic representation of the genetic constitution of the egg cytoplasm, zygote nuclei and polar bodies in the various crosses referred to in the body of the paper. Any one of the three mutant genes *dor*, *fu* or *r* may be substituted for *m* which simply represents mutant, but the same mutant must be substituted throughout any one scheme. — Nuclei and polar bodies containing the mutant gene are represented by closed circles while those containing the normal X chromosome are represented by fine diagonal lines. The Y chromosome is indicated by stippling. Egg cytoplasm from the mutant females is represented by lack of shading within the oval and cytoplasm from heterozygous females by diagonal lines

in the zygote nucleus or somatic cells of the mother, just as the lethal effect of the sex-linked genes can be counteracted by the presence of their normal alleles at one of these two stages.

POULSON (1945) pointed out that *fu* in many respects offered interesting possibilities for studying the interactions of genes and cytoplasm in the course of development and differentiation. He also indicated the similarity of the action of *fu* with that of certain genes and cytoplasmic factors in *Zea* and *Paramecium*, and suggested the desirability of an embryological study.

Another reason for the study of any embryonic lethal is that such studies have added greatly to our knowledge of the genetic control of development in *Drosophila* (POULSON 1940, 1945; HADORN 1948, 1950, 1951).

Since all three sex-linked genes causing this rare type of female-sterility result in death of the zygote during embryonic development, the effects of each on embryogenesis were studied. In the case of *fu* and *dor*, embryos from crosses of mutants and from crosses of mutant females to nonmutant males were studied to determine whether there were differences in the patterns of damage (terminology suggested by HADORN 1945) and whether there were any effects on development of the heterozygous females when the father was non-mutant. It was hoped that a comparison of the effects of the three genes on embryogenesis might produce some information on the mode of action of the genes on the egg cytoplasm. The effects of each of the genes on embryogenesis will be discussed in separate papers in a series, presented in the order in which the genes express themselves during the course of embryonic development.

The first paper in the series will be concerned with the pattern of development in *dor* embryos, which become abnormal early in embryogenesis.

The normal embryology of *Drosophila* will be mentioned only briefly, but detailed descriptions may be found in SONNENBLICK (1950) and POULSON (1950).

Material and methods

The mutant phenotype. Deep orange is located at 0.0 + on the X chromosome and derives its name from its effect on eye color (MERRELL 1947). The color of the eyes varies with the temperature at which pupal development takes place (COUNCE, unpublished). At 25° C., the eyes are bright orange but at 18° C., the color deepens to a red-orange. In both instances the eyes darken with age. The temperature at which embryonic and larval development take place has no effect on eye color.

The gene also affects certain internal structures (COUNCE, unpublished). The Malpighian tubules of the adults are of a much brighter yellow than those of wild-type flies. Fat bodies are more conspicuous in the two-day old mutant than in normal flies of the same age. The number of egg strings in the ovary is reduced and in sectioned material, degeneration of follicles, similar to that reported by BEATTY (1949) for *apterous*[4], is frequently apparent. Examination of the freshly dissected internal genitalia of the female failed to disclose any other structural abnormalities.

Stocks. The *dor* stock was originally supplied to the Institute of Animal Genetics by Dr. MERRELL. *Oregon K* flies used as controls and as parents in certain crosses with the mutant came from the stock maintained at the Institute. All flies were raised on the standard cornmeal-agar medium.

Because *dor* is sex-linked, it was balanced against the *CIB* chromosome. Flies were maintained in ordinary stock bottles and kept at 18° C. or at 25° C.

Egg collections. For the collection of eggs, forty to sixty mutant females three to five days of age were used. These had been mated the day before. Twenty pairs of *Ore K* flies gave an adequate number of eggs. Flies were placed in half-pint milk bottles inverted over watch glasses containing a 3 percent agar solution to which 1 percent acetic alcohol had been added. The surface was shaved off to provide a rough area. Yeast was not placed on the discs but if eggs were to be collected from one batch of females on more than one day, the flies were placed overnight in bottles containing extra yeast. No batch of females was used for more than four successive days, so that the mothers of all embryos studied ranged in age from four to nine days. All egg collections were made at 25° C.

Usually eggs were collected over a one hour period and the age of embryos could thus be determined within one half-hour. In some instances when early stages were desired, the collection interval was shortened to 30 or 15 minutes. The first collection of eggs was discarded since eggs may be retained in the uterus for varying lengths of time following fertilization, especially when females are not laying rapidly. All eggs were kept in moist chambers at 25° C. until the desired developmental stage had been reached.

Observations on living eggs. Observations of living eggs were made under the high powers of the microscope after removing the chorion by mechanical means (POULSON 1937) and mounting the eggs in hanging drop slides. It was noticed when eggs were placed in the formol:alcohol:acetic acid fixative (F.A.A.) used, the chorion was rendered transparent to reflected light. As embryos are capable of surviving for a short period in this fluid if embryonic membranes are intact, this characteristic was utilized for studying the gross appearance of living embryos, using a binocular dissecting microscope.

Fixation, embedding and staining. The procedures of fixation, dehydration and infiltration are those recommended by SMITH (1940) for animal eggs, which BEATTY (unpublished) found especially suitable for *Drosophila* eggs. In order to insure rapid and even fixation it is necessary to prick the vitelline membrane and for this purpose, needles of tungsten wire sharpened to a fine point in fused sodium nitrite were used (PANTIN 1948). The chorion was not removed as it does not hinder fixation and the chorionic filaments are useful guides for orientation during embedding and also in determining orientation of sectioned material. Embedding was done in hard wax, and sections cut at 3—5 microns.

The majority of sections were stained with Heidenhain's iron hematoxylin according to the schedule suggested by Sonnenblick (1950). Some were counterstained with eosin and a few embryos were stained by the Feulgen method.

Ovaries were fixed in F.A.A. or Bouin's and dehydrated using the butyl alcohol series (Smith 1940). Sections were cut at 10 microns and stained with iron hematoxylin and eosin. Whole mounts of ovaries were prepared using the method suggested by Beatty (1949). Fresh internal genitalia were dissected and examined in insect saline (0.75% NaCl).

Abnormal embryogenesis in *deep orange* embryos

Matings of mutant to mutant

Merrell (1947) dechorionated and examined 100 eggs from a cross of mutant females to mutant males and found that no characteristic stage of development was reached by the zygotes. All stages of embryonic development were found and two larvae were even observed, but "... in nearly all cases the differentiation appeared to be abnormal".

Merrell's observations could not be confirmed by us. Although the lethal embryos could be divided into two fairly discrete groups, within each group the pattern of damage was quite consistent; in fact, more consistent than is usual in lethal embryonic mutants. There are several possible explanations for this difference. Although the Edinburgh *dor* stock was originally obtained from Dr. Merrell, it was almost lost through contamination and the stock used in this work was reestablished from a very few flies. This may mean that the genetic background in this stock was considerably modified. It has been shown in several instances (Hadorn 1940, 1951; Hadorn and Gloor 1943; Gloor 1945) that the expression of lethality — indeed, the "phase specificity" (Hadorn 1949) of its action — is markedly affected by the genetic milieu. It is also possible that the expression of the gene has become modified in the course of time. This is not unknown in *Drosophila* (cf. Bridges and Brehme 1944; examples are the genes *Blond* and *bent*[3]).

In 65 percent of the embryos from the cross of homozygous *dor* females to *dor* males, maturation divisions are abnormal or development ceases during the early stages of cleavage. These will be referred to as "early lethals". Those embryos which successfully pass this critical stage continue to develop for some hours, but blastoderm formation and subsequent gastrular movements are abnormal. Cellular degeneration in the latter group, hereafter referred to as "late lethals", sets in as early as four and one half hours after fertilization, although some tissues are differentiated after this time, and in a few instances, some organs may form.

The oöcyte. Of special interest because of the maternal nature of the action of the mutant gene are abnormalities in the egg prior to sperm entry.

Meagreness of cytoplasm and yolk is quite obvious in the mature oöcyte of *dor* females. Frequently oöcytes in the same ovary will contain quite different amounts of oöplasm (Fig. 2).

Although the periplasm is present in the developing oöcyte, only one of the abnormalities which have been observed in the structure of the periplasm is obvious at this time. This is the extreme shallowness of the periplasm at the poles. Differentiation of the polar granules at the posterior pole is apparently normal, but the rapid degeneration of the pole cells following their formation

later in development may indicate that submicroscopic organization of the posterior polar plasm is abnormal. Disintegration of the pole cells may of course be due to the small amount of cytoplasm available to the nuclei as a result of the shallowness of the polar plasm but other nuclei which receive less than the normal amount of cytoplasm generally do not show signs of degeneration until later in development.

Other abnormalities in the periplasm become obvious only after deposition of the egg. The abnormal breadth of the dorsal periplasm, especially in the anterior half, becomes obvious shortly after the egg is laid (sometimes before the gamete nuclei have fused) while the absence (breakdown?) of the periplasm in restricted regions on the ventral surface can be observed only at the time of migration of nuclei. It is possible, of course, that earlier abnormalities on a biochemical level which cause these visible manifestations of irregularity are established during the period of oöcyte formation.

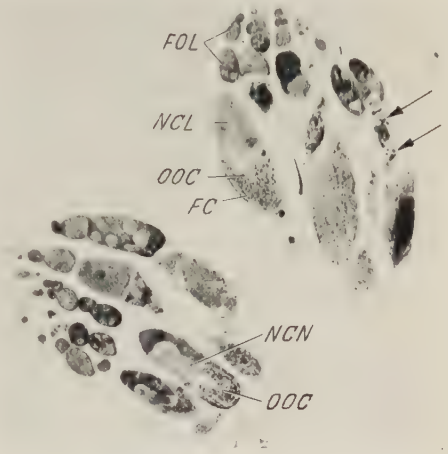

Fig. 2. Section through the ovaries of a *dor*/*dor* female. The contents of the nurse cells may be seen emptying into two developing oöcytes. Arrows point to degenerating follicles. Differences in yolk content of mature oöcytes are apparent. 45 ×. *FC* follicle cells; *FOL* follicle; *NCL* nurse cell; *NCN* nurse cell nucleus; *OOC* oöcyte

Maturation. The first maturation division is apparently normal. Characteristic of eggs which cease development during the second maturation division are large, distorted multipolar spindles on which the chromosomes are scattered helter-skelter (Fig. 3). These abnormal spindles are assumed to be maturation spindles for two reasons. Firstly, no centrioles have been identified at any pole of the spindle configuration, a characteristic of the maturation figures in normal eggs. Secondly, the typical metaphase plate formed by the polar body nuclei after syngamy has taken place is not found in these eggs, although it is present in other *dor* embryos in which developmental abnormalities become apparent at the time of the first cleavage division.

The anterior end of the egg, and especially the dorsal region, consists of a mass of cytoplasm with little or no yolk. Characteristically there is an extremely spongy region of cytoplasm in the region of the abnormal spindle which stains less heavily than the rest (Figs. 3, 4, cf. Fig. 10, Sonnenblick 1950, which shows appearance of normal egg). This may correspond to the nucleoplasm of the germinal vesicle. This massive accumulation of cytoplasm is not obvious at first, although in eggs fixed fifteen minutes after deposition there is a slight indication of a thickening of the dorsal periplasm in the anterior end. The accumulation progresses, and by $4^1/_2$ hours, the distribution of extra-nuclear material is markedly abnormal, most of the cytoplasm having accumulated anteriorly

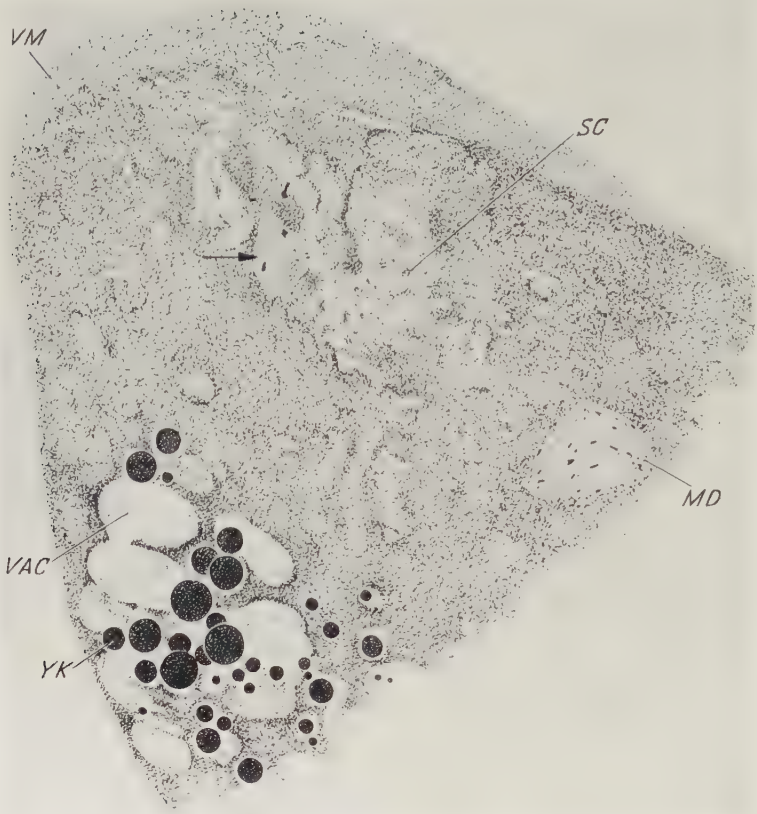

Fig. 3. Drawing of longitudinal section of "early lethal" $4^1/_2$ hours of age. This embryo ceased development prior to the completion of the second maturation division. In the anterior end the arrow points to a sperm spindle (the centrioles have been omitted in the drawing). The anterior dorsal accumulation of cytoplasm is marked, and the ventral cytoplasm is extremely vacuolate. *MD* maturation division; *SC* spongy cytoplasm; *VAC* vacuole; *VM* vitelline membrane; *YK* yolk globule

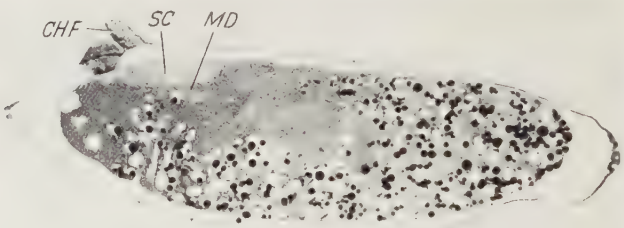

Fig. 4. Longitudinal section of $4^1/_2$ hour *dor* "early lethal". The abnormal distribution of oöplasmic materials is obvious. Note the lightly staining region of cytoplasm above the maturation figure. 170 ×. *CHF* chorionic filament; *MD* maturation division; *SC* spongy cytoplasm

while the posterior region consists almost entirely of yolk and some extremely vacuolate and stringy cytoplasm (Fig. 4).

In the egg shown in Fig. 3, a sperm spindle may be seen very near the anterior end. Abnormalities in the mechanics of division are apparent. There is

some indication that abnormal movement of sperm occurs, perhaps as a result of the abnormal accumulation of cytoplasm in the anterior end, for in many embryos which cease development early, sperm spindles are found abnormally near the anterior end of the egg. In one such egg, five sperm spindles were found at the anterior tip.

In many eggs, the abnormal maturation spindle degenerates and fragments of chromatin become distributed throughout the egg. In others, nuclei of various sizes are formed, some containing only one chromosome. All eggs which cease development at this stage degenerate rapidly, the formation of spherical droplets of cytoplasm which is characteristic of unfertilized eggs beginning about six hours after deposition. The term "unfertilized" is used here to mean that fusion of gamete nuclei to form a zygote nucleus has not taken place. Many eggs have been observed in the course of this work and in the work of others (Fahmy 1952) in which fusion of gametes has not occurred although sperm have entered the egg and we propose the term "unfertilized" for such eggs, as opposed to "uninseminated" for eggs in which no sperm are present. By seven hours degeneration is so advanced in *dor* eggs which are early lethals, that it is not possible to determine whether the second maturation division has been abnormal or whether development has ceased after fusion of gamete nuclei.

Cleavage. In about one quarter of the early lethals, development becomes abnormal during the first cleavage division (Table 1). In these eggs, abnormal spindles or clumps of nuclei of varying sizes are found in a cytoplasmic island near center of the egg, the position in which the first cleavage division takes place in normal zygotes. In such lethal eggs the polar body nuclei form a large metaphase plate in the anterior dorsal region. There is no indication of abnormal numbers of chromosomes in the polar body nucleus, implying that the female pronucleus contained at least the normal number of chromosomes.

Abnormalities in later cleavages ensue in some embryos. Multipolar spindles or abnormal spindles are commonly found (Fig. 5). Clumped nuclei, nuclei of various sizes and polyploid spindles may also be present (Fig. 6). Synchrony of nuclear divisions is not always maintained. Normal and abnormal spindles and nuclei may be found in the same embryo.

The formation of cytoplasmic islands around cleavage nuclei may also be affected. Although in normal embryos, each cleavage nucleus is surrounded by its own cytoplasmic island, such discrete islands are rare in *dor* embryos. Many of the cytoplasmic islands are small whether they contain one or several nuclei, probably as a result of the general paucity of cytoplasm, a characteristic of many eggs from *dor* females.

Migration. If embryos successfully pass the early critical period, they continue to develop for several hours, although the events leading to blastoderm formation and gastrulation are abnormal.

Migration of nuclei to the surface is abnormal in many *dor* embryos which survive the early critical period. Clumping of nuclei, which is common, may prevent some nuclei from reaching the surface to take part in blastoderm formation. A second factor which is involved in abnormal blastoderm formation is the abnormality of the periplasm. In *dor* embryos, this outer layer of cytoplasm, which in normal embryos is uniform in thickness except at the poles, varies

greatly in width, being abnormally broad in some regions (usually dorsal) and excessively thin or non-existent in others. Nuclei never migrate into regions where the periplasm is absent. Poulson (1940, 1945) has observed the breakdown of periplasm in those regions of the *nullo-X* embryos which nuclei did not enter. It is most probable in the present instance that a breakdown occurs in restricted regions of the periplasm at a time just prior to migration since no early egg stages have been found in which regions are entirely devoid of periplasm although variations in thickness are apparent at this time.

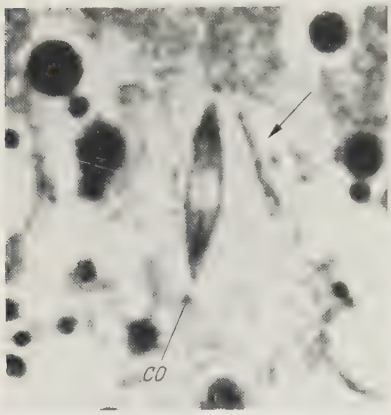

Fig. 5

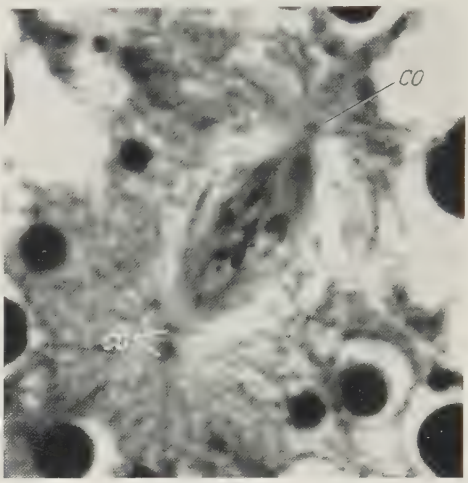

Fig. 6

Fig. 5. Abnormal cleavage mitosis from *dor* embryo. The anaphase figure is apparently normal, but the arrow points to a spindle at one side which contains a single chromosome. 1800 ×. *CO* centriole

Fig. 6. Polyploid cleavage spindle from *dor* embryo ($3^1/_2$ hours) in which all nuclei were polyploid. The centrioles have continued to divide as evidenced by the large number present at one pole. Only one of the centrioles at the other pole is apparent in this section. Lagging of chromosomes is obvious. 1800 ×. *CO* centriole

Pole Cell formation. Formation of pole cells is never normal in *dor* embryos. Because the pole cells are smaller (probably because less cytoplasm is present around the nucleus) and fewer in number than in normal embryos, they appear to adhere more closely to the main body of the embryo, and to form a less discrete group of cells (Fig. 7). Some *dor* embryos have been found in which no pole cells could be identified.

The cytoplasm of the pole cells becomes vacuolar at an early stage in their development and cellular degeneration and pycnosis may be observed shortly after the pole cells have formed. Perhaps as a result of abnormalities in the formation of the posterior midgut invagination, the pole cells in some embryos are not carried to the interior but remain at the posterior end where they degenerate.

Blastoderm formation. Abnormalities in nuclear migration and in the structure of the periplasm have been mentioned. In approximately one half of the embryos which survive the first critical period, the blastoderm cells do not completely cover the surface and there is no migration of nuclei from adjacent areas of the blastoderm to obliterate gaps (Fig. 7). These gaps are always on the ventral surface or at the poles, although their position is not otherwise constant.

The broad cytoplasmic band around the whole egg which is present at the end of migration is irregular in width and in many embryos it is very thin, especially ventrally. The blastoderm cells which form are not the typical columnar cells of the normal blastoderm but are small and round, and contain little cytoplasm. The cytoplasm in all *dor* embryos is grainy in appearance and gives a different staining reaction than that of normal embryos.

Frequently, relatively large masses of cytoplasm remain outside the cell membranes at the time of formation of the cellular blastoderm (3 hours). It is probable that formation of the inner membrane of the cell is so conditioned that the wall forms when the inward growing walls from the periphery approach the inner surface of the cell mass. If this is the case and synchrony of cell membrane

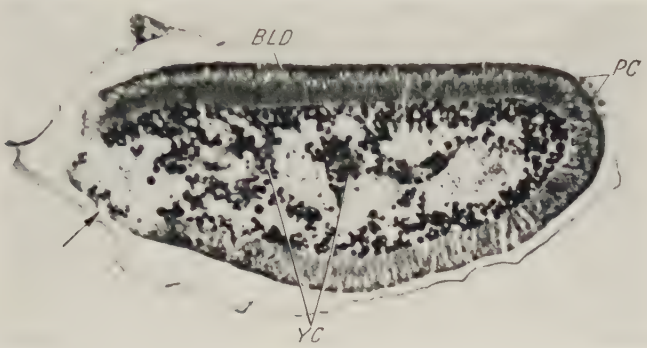

Fig. 7. Longitudinal section of *dor* embryo at the cellular blastoderm stage (3 hours). The dorsal wall is thickened and cells are completely absent in the anterior ventral region (arrow). The abnormally small pole cells, already showing indications of deterioration, may be seen at the posterior end. Large clumps of yolk cells are present interiorly. Yolk globules tend to be distributed around the periphery and only minute globules connected with cytoplasmic "strings" fill the central region. 160 ×. *BLD* blastoderm; *PC* pole cells; *YC* yolk cells

formation is maintained, the depth of the cellular layer would be determined by the region of least depth. This would not have any marked effect in normal embryos where the cytoplasmic border is about the same depth throughout, but is important in *dor* embryos where variations in the thickness of the cytoplasmic border are pronounced. The unenclosed cytoplasm in the lethal embryo is later incorporated into the primitive gut or yolk sac.

Yolk. Abnormalities in the distribution of yolk in *dor* embryos become apparent in the blastoderm stage (Fig. 7). Yolk globules are not distributed evenly throughout the central region of the embryo but are concentrated at the periphery, the innermost region being devoid of large globules although minute droplets of material connected by cytoplasmic strings are found there. Abnormally large globules are frequently observed as are disintegrating ones. Yolk nuclei are frequently clumped together.

Gastrulation. Gastrulation is abnormal in all *dor* embryos which develop beyond cleavage stages. This is not due to inherent abnormalities in gastrulation processes themselves but can be traced back to abnormal blastoderm formation.

Because of the abnormal structure of the ventral wall, formation of the ventral furrow fails to take place or is abortive. An attempt at invagination of the furrow takes place however in those regions where sufficient cellular material is available and a germ band of sorts is formed, although it is restricted in size.

The cephalic furrow usually forms. The posterior midgut forms but the cells making up the invagination are not normal in shape. Instead they are small and round and contain little cytoplasm. The posterior midgut invagination rarely moves to the anterior position which it attains in normal embryos. Most frequently it deepens and elongates from the posterior pole of the embryo, eventually filling the posterior third with its mass (Fig. 8). Rarely some anteriorward movement occurs in embryos in which a partial inner germ band has formed posteriorly.

The pole cells, when they are included in the posterior midgut invagination, already or soon show signs of degenerative change. Migration of the pole cells from the posterior midgut invagination to the body cavity never occurs. The

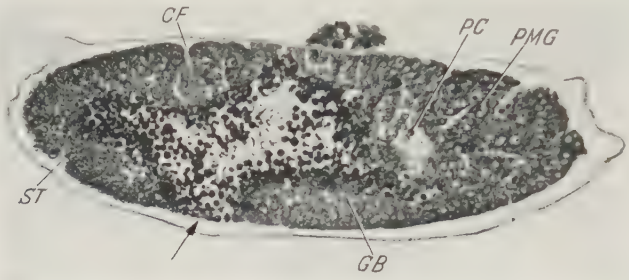

Fig. 8. Longitudinal section of *dor* embryo at the end of gastrulation (6 hours). The posterior midgut has invaginated from the posterior region and the pole cells remain within it. The cephalic furrow, stomodaeum and a rudimentary germ band are also present. The arrow points to a gap in the ventral wall, the result of abnormal blastoderm formation. 160 ×. *CF* cephalic furrow; *GB* germ band; *PC* pole cells; *PMG* posterior midgut invagination; *ST* stomodaeum

abnormal position of the posterior midgut invagination is probably not responsible for the failure of migration since other embryos have been found in which the posterior midgut has invaginated from the posterior region without preventing the migration of the pole cells through the gut wall into the body cavity (Counce, unpublished material). On the other hand, Aboim (1945) has recorded the passage of dying pole cells through the gut into the body cavity so we cannot assume that degenerative changes *per se* are responsible for the failure of these pole cells to migrate.

Further development. Although pole cells begin to degenerate almost as soon as they have formed, other cells seem capable of dividing for a considerable period. However, the initial lack of cytoplasmic materials is never overcome and as the cells continue to divide, they become smaller and smaller. By the end of gastrulation ($4^1/_2$ hours) cells at the surface and some cells in the interior, especially those of the posterior midgut rudiment, begin to degenerate. Abnormal mitotic figures are found as well as polyploid and multinucleate cells. Some multinucleate cells are found in which one nucleus is polyploid and the others apparently diploid.

In those embryos which apparently have a more nearly adequate supply of cytoplasm, histogenesis and some organogenesis take place. Functional salivary glands form in some embryos and the presence of scattered muscle cells indicates that a partial germ band has formed. In all embryos in which histogenesis takes place, differentiation of nervous tissue into fibers and ganglion

cells occurs. While these processes of differentiation are going on, degeneration continues, being most marked at the surface and at the poles (Fig. 9). By 18 hours, degeneration of the anterior and posterior regions is so marked that their cellular nature has been obliterated, and the anterior end is filled with an amorphous mass of lightly staining material. By this time, most of the cells in the interior have begun to degenerate as evidenced by the presence of pycnotic nuclei and vacuolate or darkly staining cytoplasm.

All lethal embryos which die during the second critical period of development retain an outline reminiscent of the gastrular outline of normal embryos.

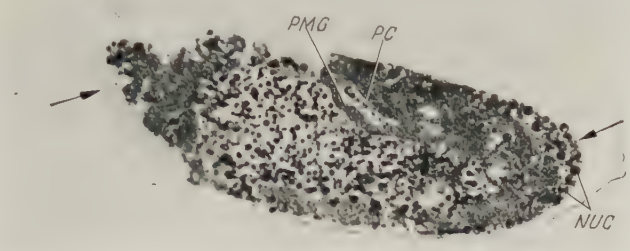

Fig. 9. Longitudinal section of *dor* "late lethal" at 14—16 hours. Cells in the anterior and posterior regions have completely disintegrated (arrows) and almost all cells at the surface are deteriorating. These appear as black objects in this figure. Degeneration of the pole cells is advanced. The outline resembles that of the late gastrula stage in normal embryos. 160 ×. *NUC* nuclei; *PC* pole cells; *PMG* posterior midgut invagination

Matings of dor females to Ore K males

In general, the pattern of damage in lethal embryos (*dor*/*Y*) in these crosses is like that observed in embryos when both parents are of the mutant genotype. Critical developmental abnormalities occur either during maturation divisions or early cleavage, or embryos develop beyond this stage only to die as the result of abnormal blastoderm formation and subsequent abnormalities in gastrulation. However differences are apparent in the distribution of the two groups of abnormal embryos from the two kinds of matings. A possible maternal effect of the gene on the embryonic development of the heterozygous female has also been observed.

Table 1. *Time of cessation of development in early lethals from dor females**

dor females mated to:	Number of embryos ceasing development at:			
	Meiosis	1st cleav.	2nd—3rd cleav.	Later cleav.
dor/*Y* ♂♂	21 (62.3%)	8 (23.2%)	2 (5.8%)	3 (8.5%)
+/*Y* ♂♂	5 (20.8%)	8 (33.3%)	5 (20.8%)	6 (25.0%)

Maturation. Abnormalities in meiosis are relatively rare in embryos from crosses where the father is of the non-mutant genotype (Table 1). Although the dorsal anterior accumulation of cytoplasm also occurs in eggs from crosses of this type, it is neither as rapid nor as marked as in embryos from mutant parents.

* Based on examination of eggs 3 to $4^1/_2$ hours of age at which time it is possible to determine accurately the stage at which development has ceased. With younger embryos, one cannot be sure that development has really ceased while in older embryos deterioration makes it difficult to ascertain exactly the stage at which development has ceased.

The "spongy" regions of the cytoplasm in the vicinity of the abnormal spindle, which are so characteristic of the same group of embryos from crosses of mutant to mutant, are not found in many of these embryos. Aberrant meiotic figures are similar in embryos in both crosses.

Cleavage. Abnormalities in cleavage are like those observed in embryos from mutant parents. One of the more interesting embryos in this category was one in which all cleavage nuclei (16) were polyploid and the number of centrioles at least doubled. Although in many *dor* lethal embryos, some polyploid nuclei

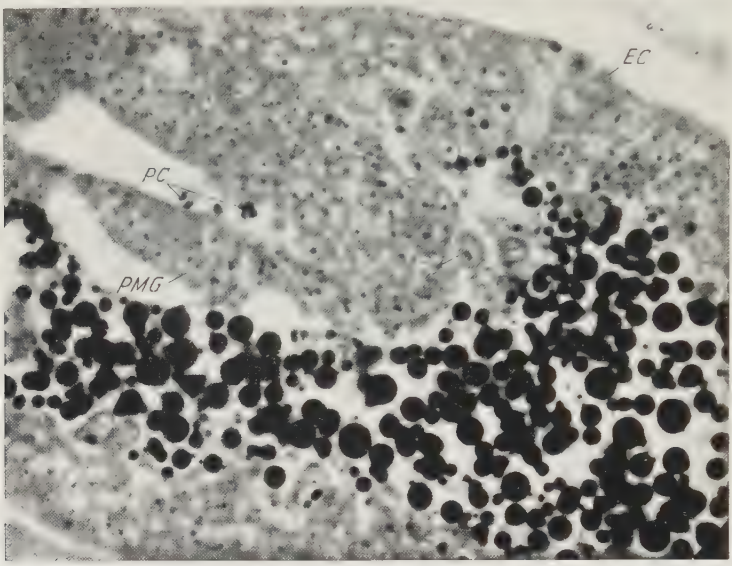

Fig. 10. Longitudinal section of an embryo 8 hours of age from a *dor dor* female mated to an *Ore K* male. The embryo is normal in all respects with the exception of some of the pole cells. Some of these pole cells have migrated to the outside of the posterior midgut invagination and are apparently normal, but the nuclei of those pole cells still within the invagination show signs of pycnosis. 500 ×. *EC* ectoderm; *PC* pole cells; *PMG* posterior midgut invagination

are found, no other was found in which all nuclei were polyploid. In this one embryo it is probable that at the end of the fourth cleavage division the resultant nuclei did not separate completely. When the spindles formed at the next division, they were so near that the spindles and chromosomal complements of the two became incorporated into one large division figure. A less likely explanation is that polyploidy was established at the beginning of the second cleavage division and that the centrioles although doubled in number, were able to function in a relatively normal fashion in successive mitoses.

In some embryos in which cleavage nuclei are apparently normal, and surrounded by discrete cytoplasmic island the paucity of cytoplasm as compared with normal eggs is striking.

Subsequent development. No striking differences in pattern of damage between these embryos and the late lethals from mutant parents are apparent. A possible maternal effect of the *dor* gene on the female embryo (*dor*/+) has been observed. Abnormal pole cells with vacuolate cytoplasm and pycnotic nuclei are found in

the posterior midgut invagination of some embryos after others have migrated into the body cavity (Fig. 10). These embryos are normal in all other respects and have developed far beyond the stage at which degeneration of all tissues has begun in lethal embryos.

Composition of the two lethal groups in embryos from matings of mutant to mutant and of mutant females to non-mutant males

All sectioned embryos three hours of age or older were classified as normal, late lethals (blastoderm formation and gastrulation abnormal) or early lethals (failure of development prior to migration of nuclei) (Table 2). No sections of younger embryos were included because in some instances it was not possible to ascertain that development had really ceased, and in others, whether minor abnormalities such as clumping of small groups of nuclei were sufficient to cause abnormal development of the whole embryo.

Table 2. *Distribution of early and late lethals in crosses of mutant females to mutant males and of mutant females to non-mutant males*

dor female mated to:	Early lethals	Late lethals*	Normal embryos	Total
dor ♂♂	109	55 (♂♂)	0	164
Ore K ♂♂	27	66 (♂♂)	65 (♀♀)	158

Several points are to be noticed in connection with Table 2:

1. Cytological examination has indicated that all late lethals are male embryos. Therefore in crosses of mutant to mutant, all females must be included among the early lethals. This different pattern of damage in the two sexes may be the result of differential developmental rates, or of dosage effect.

2. In crosses where the father is non-mutant, the early lethals must consist of both males and females since there would be a significant deviation from a 1:1 sex ratio if the early lethals were of only one sex. This indicates that a maternal effect may result in the death of some female embryos and possibly some males.

3. Even when one takes into consideration the fact that all females are included among the early lethals in matings of mutant to mutant, the proportion of early lethals is much higher in these matings than in matings where the father is non-mutant. This may mean that the maternal effect is expressed more strongly in mutant crosses. A possible explantation is that in these crosses there are no normal X chromosomes in the supernumerary sperm, which may alleviate the condition in crosses where the father is non-mutant.

4. There is a different distribution in the stages of development reached by early lethals in the two crosses (Table 1). Over half of the early lethals in mutant crosses cease development before the first cleavage division, while only one-fifth of the early lethals die at this stage in crosses where the father is non-mutant. These "early" early lethals in mutant crosses may be females only. Unfortunately, we can obtain no cytological evidence on this point since death occurs before a zygote nucleus has formed, and these are gametic lethals in a sense.

* Cytological examination by Dr. B. M. Slizynski of neuroblast mitoses in smears of late lethals indicated that all late lethals were males regardless of the genotype of the father.

Discussion

Maternal effects. The most obvious of the maternal effects of the mutant gene is the effect of the genotype of the mother on the viability of offspring (cf. Fig. 1). In matings of a heterozygous female to a mutant male, the presence of a normal X chromosome in the somatic cells of the mother is sufficient to counteract the effect of the gene in homo- and hemizygotes. This means that the presence of the gene itself in a homo- or hemizygous condition is not sufficient to cause the death of the zygote. However, it is also apparent that the gene acting through the homozygous mother can so influence the cytoplasm of the egg prior to fertilization that it may be lethal to the zygote unless the male gamete nucleus furnishes the normal allelomorph of the gene. In some instances even the presence of the normal X chromosome is not sufficient to overcome this "maternal" effect.

Lynch (1919) was the first to attempt an explanation for the unusual sterility effects of genes of this type. She assumed that development of mutant zygotes from heterozygous mothers might be due to the action of the normal allele in two of the three polar bodies in the egg. However, there is an attractive alternative to this explanation now available in the light of more recent knowledge. In *Drosophila* the egg cytoplasm and yolk globules are formed as a result of the activity of fifteen nurse cells in each follicle which nourish the developing oöcyte. (For detailed descriptions of egg formation in *Drosophila* see Guyénot and Naville 1933; Stolfi 1938; Miller 1950). During the later stages of oöcyte development, the contents of these nurse cells are literally poured into the egg (Fig. 2), including an enormous amount of nuclear material, for the nuclei of the nurse cells at this time are highly polytenic (Painter and Reindorp 1939). (In the eggs of some female-sterile mutants, whole nurse cell nuclei have been observed in the oöplasm — Beatty 1949, Counce, unpublished material.) In eggs produced by a heterozygous female therefore, the cytoplasm of the egg would contain a considerable amount of normal X nuclear material.

The possible significance of this for the maternal effect considered here is obvious. If the developmental system which is affected by the mutant gene is one which first becomes active in early stages of embryogenesis, a sufficient amount of normal X substance may be present in the cytoplasm to by-pass or overcome the deleterious effects of the genes in the zygote nucleus. We have no proof of this contention as far as these female-sterility genes are concerned. However, Painter (1940) in seeking to explain the rapid synthesis of chromosomal material which must take place during cleavage divisions cited evidence to indicate that "the derivatives of the absorbed nuclear material may persist in a partially polymerized form and . . . 'the rapid building up of the cleavage chromosomes is possible in the segmenting egg because the synthesis is more in the nature of a reassembling of already existing materials . . . under the guidance of the active chromosomes, rather than an actual synthesis of the building blocks from relatively simple substances'" (as quoted in Sonnenblick 1950).

It is of course possible that normal development in the instance of the female sterility genes is due to the combined effects of the "heterozygosity" of the oöplasm and the activity of the two polar bodies which contain the normal X chromosome.

Another of the maternal effects is evidenced in the structure of oöcytes and eggs. It has been pointed out that paucity of yolk and cytoplasm are frequently apparent in the eggs of *dor* females. Some of the embryos which died in matings where the father was not of the mutant genotype were apparently normal except for the meagreness of oöplasmic materials. This type of defect is not limited to eggs produced by *dor* females, but has been observed in eggs from *fu* and *r* mutants (Counce, unpublished material) and Beatty (1949) noted similar abnormalities in oöcytes and eggs of the female-sterile mutants *ocelliless* and *comb-gap*. Oöplasmic abnormalities however, appear in some instances to be of a consistent type in various mutants and may vary from mutant to mutant. Abnormalities in egg structure are in part responsible for the low percentage of imagines which appear in matings of the type shown in Fig. 1b. Other contributing factors to this low percentage are the many dangers inherent in any culture bottle when the number of larvae is small and molds and bacteria multiply rapidly.

A third maternal effect is that which causes some embryos to die regardless of the genetic constitution of the zygote nucleus. (See Table 2 and discussion immediately following it.) A maternal effect of this sort in addition to a lethal effect has been shown to exist in the instance of *Polyphaen*, a dominant gene in *Drosophila funebris* which is lethal when homozygous (Strasburger and Körner 1939). The maternal effect increased the number of unhatched eggs in crosses of heterozygote to heterozygote from the expected 25 percent to about 55 percent. Although no definite effect of the gene on embryonic development could be found, it was concluded on the basis of the *time* of death of embryos in crosses of heterozygotes and of mutant females to normal males, that those which died because of the maternal effect could be separated from those which died because of genetic constitution.

Presumably a maternal effect is responsible for the degeneration of some of the pole cells in heterozygous female embryos from mutant mothers (Fig. 10). However, the gene may also cause degeneration of pole cells when the mother is not of the mutant genotype since number the of eggs strings is reduced in the homozygous mutant and may be in heterozygous females, independent of the genotype of the mother. This point remains to be confirmed.

The possible mode of action of dor in embryogenesis. We must now seek an explanation for the manifold effects of the *dor* gene during the course of embryonic development. We will attempt to show in the following discussion that the evidence points to a primary effect of the gene on the physical and/or chemical nature of the cytoplasm.

In those embryos in which development becomes abnormal at the time of the second maturation division, certain characteristics are evident. The maturation spindle is grossly distorted, sperm heads and spindles may be found in unusual locations and the eventual aberrant distribution of oöplasmic materials is constant and characteristic.

Two possible explanations for the formation of abnormal maturation spindles present themselves if we interpret the basic abnormality in the eggs as an abnormal condition of the cytoplasm. One is that the abnormal condition of the cytoplasm itself is responsible for aberrant spindle formation and hence abnormalities in the mechanics of meiosis. The other is that the sperm are prevented

from moving normally through the cytoplasm because of the nature of the cytoplasm or because of abnormal distribution of oöplasmic materials, since the failure of maturation divisions is accompanied by abnormal movement of most of the cytoplasm to the dorsal anterior regions of the egg. There is evidence to indicate that sperm do not penetrate deeply into *dor* eggs, as sperm heads and sperm nuclei have been observed just inside the anterior tip of the egg. The failure of sperm to reach the region of the maturation divisions may cause some disturbance in the normal synchrony of the divisions and result in formation of a spindle or spindles incorporating the chromosomal elements of all four female nuclei.

If we assume that the normal allele of *dor* is responsible for the initiation of changes in the nature of the cytoplasm which are necessary for the completion of the maturation divisions, the movement of sperm and the redistribution of oöplasmic constituents, an effect of the gene prior to formation of the zygote nucleus can be explained. Glass and Plaine (1950) have cited evidence which indicates that at least one other gene in *Drosophila* becomes effective at the moment of sperm entry. It seems likely that events of the precise nature of those leading to syngamy in *Drosophila* are under genetic control just as are those events which occur in the course of development.

An effect of the gene prior to actual pronuclear fusion may also be invoked to explain differences in distribution of oöplasmic materials which are apparent between early lethals in which the father is mutant and those in which the father in non-mutant. Because of polyspermy (which regularly occurs in *Drosophila*, Sonnenblick 1950) in matings of the latter sort, the presence of some sperm with normal X chromosomes might for a time counteract the effects of the mutant gene to the extent that some of the abnormal movement of cytoplasm could be overcome. However, the effect would not persist unless the male pronucleus contained the normal allele.

Various cytological abnormalities observed in embryos which cease development during cleavage divisions could easily be related to cytoplasmic abnormalities affecting spindle formation and subsequent separation of nuclei.

Another characteristic of spindle formation in *dor* embryos which points to cytoplasmic abnormalities may give some hint as to the possible physical causes underlying these abnormalities. Dr. Slizynski pointed out that the spindle fibres of all division figures in sectioned *dor* embryos are much more obvious than those in normal embryos. Huettner (1933) observed in his sectioned material that those spindles nearest the point at which the egg had been punctured to allow fixation were always better defined than other spindles in the egg. He attributed this phenomenon to the release of tension in the oöplasm in the region of fixation. This leads one to speculate that the tension of the oöplasm may be abnormal (less than in normal eggs ?) throughout the entire *dor* egg. This abnormal condition could be the result of abnormalities in the formation of the oöplasmic material in the mutant or of the failure of normal physical changes to take place after the sperm have entered the egg (processes which may be under the control of the normal counterpart of the mutant gene).

In sectioned material the staining reaction with iron hematoxylin is quite different for *dor* embryos than it is for normal embryos and other embryonic

lethals. The microscopic structure of the cytoplasm is also quite different from that of normals, tending to be less homogenous and more vacuolate.

Abnormal migration and aberrant blastoderm formation may also be associated with abnormal cytoplasmic conditions. At the time of gastrulation, prior aberrant blastoderm formation causes many abnormalities in mechanical processes. Eventually the cells of the embryo, which are never able to overcome the initial paucity of cytoplasm, succumb, probably as a result of discrepancies in the ratio of nuclear to cytoplasmic materials.

Other genetic factors have been described which affect early division phenomena, but in the case of the *cross-over suppresor* gene in *D. subobscura* (FAHMY 1952) and *Gowen's gene* (GOWEN 1933), the basic effect is on chromosomal rather than cytoplasmic factors. The causal mechanism behind the appearance of abnormalities in meiosis associated with the gene *claret* in *D. simulans* (WALD 1936) has yet to be explained but it is not unlikely that cytoplasmic factors have an important role.

Of special interest is KAUFMANN's study (1940) of the nature of the sterility of hybrids between *D. miranda* and *pseudoobscura*. In hybrid females, fertile eggs are produced but the course of embryonic development is grossly aberrant, both meiotic and mitotic divisions being abnormal. He interprets the sterility as genic in nature, and attributes the abnormalities to "a maternal effect of the hybrid chromosome complement on the egg cytoplasm prior to fertilization". The parallel between this and the situation in *dor* is immediately apparent.

Meiotic and mitotic abnormalities early in development have also been produced by experimental means. HOWLAND (1941) has described the occurrence of such abnormalities following centrifugation at early stages, while COUNCE and SELMAN (1955) have obtained mitotic abnormalities following ultrasonic treatment during cleavage stages. In both instances, the authors have postulated a close affinity between these abnormalities and changes in the consistency and arrangement of the cytoplasm.

Two other interesting facets of the mode of action of *dor* have been briefly mentioned. One is the difference of expression of the pattern of damage in the two sexes, which may be a dosage effect or which may result from differential developmental rates. The other is the indication of a possible role of supernumerary sperm in the early stages of embryogenesis. Polyspermy is the rule not only in *Drosophila* but in many other insects as well. Since there is now evidence at hand which indicates at least that genes may come into play before a zygote nucleus is formed, it does not seem unlikely that supernumerary sperm may have a role in development. In a later paper, more information will be presented regarding the possible role of these sperm. However, we can say that an effect of such sperm is likely, and such an effect may be invoked to explain or partially explain some rather vexing genetic problems such as penetrance and expressivity in certain mutants.

A general discussion of the effects of *dor* as compared with the effects of *fu* and *r* will be found in the third paper of this series as will a discussion of the implications of these findings and further investigations of the nature of this type of female-sterility.

Summary

1. The effect of the female-sterility gene *dor* on embryogenesis has been described.

2. In matings of *dor* females to *dor* males, female embryos cease development during maturation or early cleavage. Male embryos go on to form a blastoderm but it is aberrant and subsequent developmental events are abnormal. Cellular degeneration begins before the completion of gastrulation, and by sixteen hours the disintegration of the embryo is almost complete. Some embryos die early in development regardless of sex, probably as the result of a maternal effect.

3. The pattern of damage in male embryos from matings of *dor* females to non-*dor* males is in general like that of males from matings of mutant to mutant. A small number of embryos succumb because of a maternal effect, regardless of sex. Disintegration of pole cells contained within the posterior midgut has been observed in some female embryos.

4. The abnormalities which are associated with the gene are probably related to primary abnormalities in the cytoplasm.

5. The possible role of supernumerary sperm in the course of development is discussed.

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STUDIES ON FEMALE-STERILITY GENES IN *DROSOPHILA MELANOGASTER*

II. THE EFFECTS OF THE GENE *FUSED* ON EMBRYONIC DEVELOPMENT*, **

By

S. J. Counce

With 9 figures in the text

(Eingegangen am 11. November 1955)

Introduction

Among the most interesting of the female-sterility genes in *Drosophila melanogaster* are those which cause complete sterility only when the male parent is of the same genotype as the female. Three of the genes — *deep orange (dor)*, *fused (fu)*, and *rudimentary (r)* — are sex-linked; in addition, in each of the three the sterility is the result of embryonic death of zygotes.

The present paper is one of a series describing the effects of these genes on embryogenesis. The first in the series describes the effects of *dor* on development. this paper the effects of *fu*, while the third will deal with the effects of *r*.

Fused is located at 59.5 on the X-chromosome. Veins L3 and L4 are fused from the base of the wing to the region of the anterior crossvein which is eliminated as is the cell basal to it. The veins are also fused the tip of the wing. The ocelli are reduced or absent, and the eyes small and rough (Bridges and Brehme 1944).

Lynch (1919) was the first to describe the unusual sterility effects of the gene. She found that the *fu*/*fu* female laid eggs which appeared to be normal. However, these eggs failed to produce any adults when fertilized by sperm from *fu* males or Y sperm from non-*fu* males, but if the eggs were fertilized by an X sperm from a non-*fu* male, some adult heterozygous females were produced. Egg production in *fu* females was reduced because fewer eggs matured in the ovaries. She further observed that many of the eggs which failed to hatch contained embryos which in many respects resembled first instar larvae.

Transplantation studies by Clancy and Beadle (1937) showed that the ovaries of *fu* females transplanted at larval stages into non-*fu* hosts retained their characteristic sterility. Beatty (1949) found that although the ovaries of *fu* females were small and contained few egg strings they were histologically normal. The internal genitalia of the females were otherwise normal. The flies used by Beatty produced very few eggs, and he makes no comment in his paper regarding their development.

* This paper is taken from a thesis presented to the Senatus of the University of Edinburgh in partial requirement for the degree Doctor of Philosophy.

** This work was done during the tenure of Fulbright Scholarships in 1950 and 1951, and the Dorothy Bridgman Atkinson Fellowship of the American Association of University Women in 1952.

This paper deals with the effects of the gene *fu* on embryonic development in crosses of mutant to mutant and of mutant females to non-mutant males. In addition, the effects on embryogenesis of two *fu* alleles, *fused-glider* (fu^g) and *fused-formalin food* (fu^{ff}) have been studied in an attempt to ascertain whether allelic differences in patterns of damage exist just as differences are found in the phenotypic expressions of allelic genes.

Normal development will be referred to briefly. Detailed accounts will be found in Sonnenblick (1950) and Poulson (1950). When times are given for various events in development they coincide with those given by Poulson.

Materials and Methods

The *fu* and fu^g stocks were supplied from those maintained at the Institute of Animal Genetics in Edinburgh. *Fused-formalin food* flies were the descendants of a mutant which appeared in the progeny of flies fed as larvae on formalin treated food in the experiments of Dr. Charlotte Auerbach. The symbol for the mutant is that given to it by Dr. Auerbach. *Ore K* flies which were used as controls and as parents in certain crosses were obtdained from Institute stocks. All were raised on the standard cornmeal-agar food mixture.

All the *fu* alleles were balanced against a *CIB* chromosome.

Methods of egg collection, observation of living eggs, and histological techniques are described elsewhere (Counce 1955).

Observations

Final pattern of damage in fu embryos

Twenty hours after deposition, the lethal *fu* embryo resembles in many respects a normal embryo at the time of hatching. However, even in the living embryo, several abnormalities in differentiation may be observed (Fig. 1).

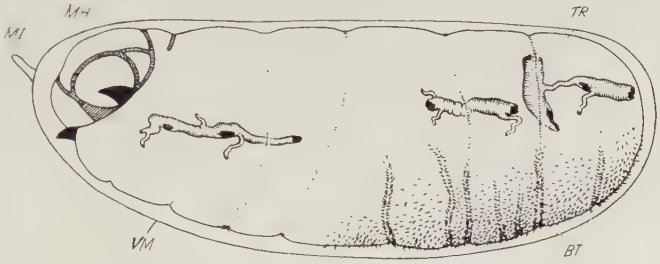

Fig. 1. Diagrammatic representation of mature *fu* embryo. Compare with Figs 1, 2 and 3 *f* and *g*. Gloor (1950). *BT* bristles; *MH* mouth hook; *MI* micropyle; *TR* trachea; *VM* vitelline membrane

The size of embryos varies greatly, some being of normal dimensions while others are much shortened. This size variation is not due to differences in the original size and conformation of the egg, but to abnormalities in growth and morphogenetic movements in the course of development.

The anterior end of the animal is blunt and covered with large areas of pigmented chitin. Segmentation is distorted. The number of segments is always less than that of normal embryos, but may vary from no visible segmentation to nine or ten segments. Individual segments are often incompletely formed,

the number apparent ventrally exceeding that dorsally. They may also vary in width and number laterally. Rings of chitinous setae, such as are found at the anterior edge of each segment in normal embryos, are only partially formed. Instead, heavy bristles are distributed more or less at random over the ventral

Fig. 2. Longitudinal section of 18½ hour *fu* embryo. The organization of the pharyngeal musculature is rudimentary. The oesophagus runs ventral to the fibres of the nervous system which should encircle it, but formation of the proventriculus is nearly normal. Partial constriction of the midgut has taken place. Segmentation is abnormal, and the nervous system is asymmetrical. 250 ×. *BR* brain; *HG* hindgut; *MG* midgut; *MP* Malpighian tubules; *MSV* visceral musculature; *MUS* somatic musculature; *OES* oesophagus; *PH* pharynx; *PMUS* pharyngeal muscles; *PV* proventriculus; *SLD* duct of the salivary glands; *TR* trachea

posterior regions, but tend to reflect the abnormal segmentation. Abnormalities in tracheal formation are apparent in living embryos after the branches have filled with air. Both anterior and posterior spiracles and the main branches of the tracheae adjacent to them are usually normal. Beyond the posterior cross-connection, however, abnormal chitinization, twisting of the tracheal stem, and absence of portions of the tracheae are apparent. The smaller branches may also show such abnormalities.

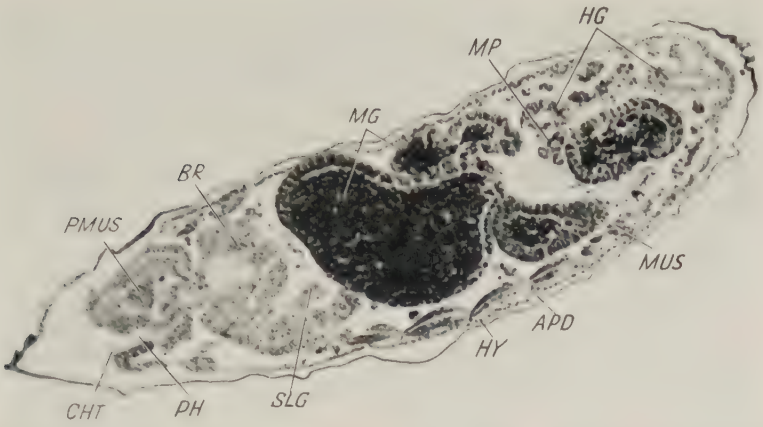

Fig. 3. Median longitudinal section of 18 hour *fu* embryo, showing the least extreme manifestation of the pattern of damage. Involution of the head is incomplete and some abnormalities in differentiation of the body musculature and in segmentation may be noted. The nervous system is only slightly asymmetrical, but has not shortened (not shown in this section). Note that the oesophagus is encircled by ganglia. 220×. *APD* apodeme; *BR* brain; *CHT* chitin; *HG* hindgut; *HY* hypodermis; *MG* midgut; *MP* Malpighian tubules; *MUS* body musculature; *PH* pharynx; *PMUS* pharyngeal muscles; *SLG* salivary gland

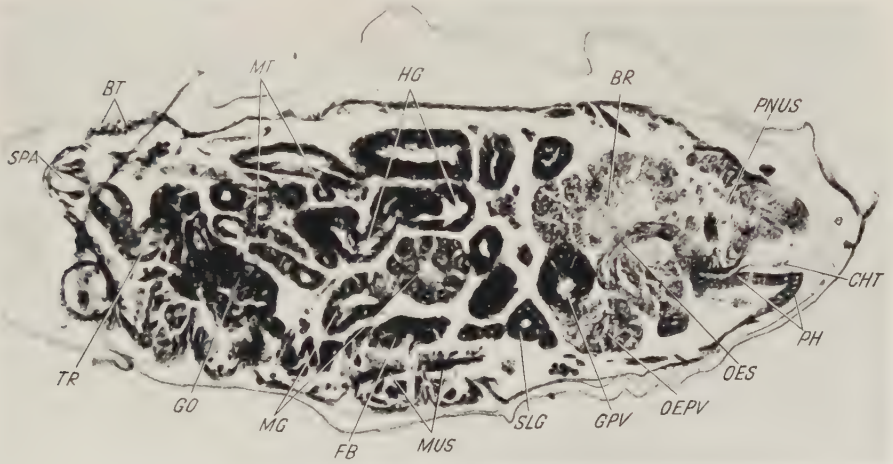

Fig. 4. Longitudinal section of 22 hour *fu* embryo showing the shortened appearance of the embryo. No segments have formed anteriorly and those which have formed posteriorly vary in width. Partial clumping of muscle cells is obvious ventrally. Differentiation of the midgut and hindgut is comparable to that in normal embryos. Union of the oesophagus and gut is abnormal resulting in abnormal structure of the proventriculus. The main branch of the tracheal system runs too near the ventral surface. The anterior end is foreshortened and differentiation of pharyngeal cells at the surface can be seen. Pharyngeal musculature is only rudimentary. The gonad has formed too near the ventral surface. 240×. *BR* brain; *BT* bristles; *CHT* chitin; *FB* fat body; *GO* gonad; *GPV* gastric portion of the proventriculus; *HG* hindgut; *MG* midgut; *MP* Malpighian tubules; *MUS* body musculature; *OEPV* oesophageal portion of the proventriculus; *OES* oesophagus; *PH* pharynx; *PMUS* pharyngeal muscles; *SLG* salivary gland; *SPA* spiracular atrium; *TR* trachea

Embryos at this stage are capable of the muscular movements associated with hatching, but these are generally less vigorous than those of normal embryos at the same stage. It has been possible to hatch a few embryos by carefully rupturing the vitelline membrane with a fine needle. These embryos may

live for an hour or more outside the membranes but never move about. The malformation of the mouth parts is so pronounced it seems improbable that hatching could occur without mechanical assistance. No larvae were ever observed in the course of this work although several hundred eggs were collected; however, Lynch (1919) reported finding two larvae.

In addition to the abnormalities which can readily be seen in living embryos, others may be observed in sectioned material (Figs 2, 3 and 4). The pattern of damage in the lethal *fu* embryo is summarized in the second column of Table I.

Abnormalities in differentiation of body musculature, the proventriculus, the gut and Malpighian tubules are apparent. The dorsal vessel is absent, and the nervous system frequently shows signs of disintegration. Differentiation of the hypodermis varies, and in some embryos both the gut and oesophageal ganglia are exposed to the surface. Differentiation of the hind gut, gonad sheath and germ cells, and fat bodies is usually normal. Embryos frequently exhibit a striking degree of asymmetry.

The way in which these various abnormalities arise in the course of development will be discussed in the following section.

Development of the pattern of damage in the fu embryo

During the course of embryogenesis, there are marked changes in the volume of the embryo as a whole, which are apparent only in the living embryo. Imaizumi (1954) has described in detail the changes in volume through early gastrulation. These and subsequent changes have been observed independently by Ede and myself in time-lapse films made of the living embryo[1].

Early in gastrulation the embryo contracts in an anterior-posterior direction (Imaizumi 1954). However, as gastrulation ends, the normal embryo again extends itself until it once more fills the vitelline membrane (Ede, Counce and Lucey 1953).

Development in *fu* embryos is apparently normal until the last stages of gastrulation. In *fu* embryos, at this time, extension is unilaterally asymmetrical, one half of the embryo extending anteriorly and the other posteriorly, almost as if a peanut were halved in the long axis, and the two halves slightly pushed in opposite directions along the long axis. These abnormal movements are probably responsible for the failure of the anterior midgut invagination to close at the surface on one side and for the asymmetrical distribution of the yolk mass and organ rudiments.

The disturbance in the bilateral symmetry which is established in all embryos by 5 to $5^1/_2$ hours is associated with many of the developmental defects which constitute the final pattern of damage in the *fu* embryo. The asymmetry of the embryo remains obvious throughout the course of development.

The developmental history of various tissues and organs in the lethal embryo will be discussed in detail.

Musculature. Ventral furrow formation, the initial step in germ band formation, generally occurs normally, although in some embryos the furrow is

[1] A film, "The Development of Drosophila" (1953) which clearly shows these movements can be obtained from the Cinematographic Unit of the Institute of Animal Genetics, Edinburgh.

Table 1. *Final pattern of damage in lethal embryos of the genotypes fu, fu^0, fu^{II}, Krüppel and Minute IV*

1.	2.	3.	4.	5.	6.
Organ System	*fu*	fu^0	fu^{II}	*Krüppel* (GLOOR 1950)	*Minute IV* Deficiency (FARNSWORTH 1951)
Body musculature.	Abnormal differentiation; asymmetric.	Slightly abnormal differentiation; asymetric.	Slightly abnormal differentiation.	Reflects abnormal segmentation.	Incomplete differentiation.
Visceral musculature.	Usually normal.	As *fu*.	As *fu*.		
Fat bodies.	Normal in most.	As *fu*.	Normal.		Reduced in size or absent.
Dorsal vessel.	Absent.	As *fu*.	As *fu*.	Normal in most.	Absent.
Nervous system.	Asymmetric; degenerates in older embryos.	As *fu*.	Some asymmetry; usually degenerates in older embryos.	Ventral nerve cord abnormal; degenerates in regular pattern.	Abnormal ventral nerve cord degenerates in regular pattern.
Tracheal system.	Abnormal in middle regions; anterior and posterior normal.	As *fu*.	Abnormal in middle regions; frequent abnormalities in posterior spiracle.	Abnormal in middle regions; posterior region normal	Normal.
Hypodermis.	May be incomplete. Abnormal bristle differentiation and distribution.	As *fu*.	Complete.	Normal.	Normal.
Segmentation.	Abnormal.	As *fu*.	Abnormal in 50% of embryos.	Abnormal.	Abnormal.
Involution.	Abnormal and incomplete.	As *fu*.	As *fu*.	Abnormal.	Involution fails or is incomplete.
Mouths parts; cephalopharyngeal apparatus.	Abnormal and foreshortened.	As *fu*.	As *fu*.	Mouth parts in abnormal position.	Structure of cephalopharyngeal apparatus abnormal.
Foregut.	Position abnormal.	As *fu*.	As *fu*.	Normal.	Absent.
Proventriculus.	Abnormal position; differentiation incomplete.	Abnormal position and incomplete differentiation in most.	Abnormal position and incomplete differentiation in most.	Abnormal position.	Absent.
Midgut.	Development retarded; may be abnormal. Walls Fragile.	Usually normal.	Usually normal.	Usually normal.	Development retarded; walls fragile.
Hindgut.	Normal.	As *fu*.	As *fu*.	Normal.	Normal.
Salivary glands.	Asymmetric; differentiation normal in most.	As *fu*.	Asymmetric; usually degenerate.	Normal.	Normal position affected by abnormal head involution.
Malpighian tubules.	Usually abnormal.	Slightly abnormal in most embryos.	Usually normal.	Absent.	Normal.

Table 1 (Continuation)

1.	2.	3.	4.	5.	6.
Organ System	*fu*	fu^{g}	fu^{tf}	*Krüppel* (Gloor 1950)	*Minute IV* Deficiency (Farnsworth 1951)
Gonads.	Asymmetric; cells usually normal; may degenerate in older embryos.	Asymmetric. Cells normal.	Asymmetric. Cells degenerate.	Usually normal.	Normal.
Yolk.	Large amounts in gut; frequently found in haemocoel.	Large amounts in gut.	Little or none in gut.	Large amounts in gut.	Large amounts in gut; some in haemocoel.

reduced in size at the time of formation. By $5^1/_2$ hours, the asymmetry in the presumptive mesoderm is very obvious, and is usually manifest as a crowding of the mesoderm to one side. By 8 to $8^1/_2$ hours,when mesodermal segmentation becomes obvious, variations in the width of the mesodermal elements may be observed. External segmentation in normal embryos becomes apparent at about 8 hours; in *fu* embryos, the time of appearance varies from 8 to 10 hours, and segmentation is abnormal from the time it is visible (cf. Fig. 4). Some of the abnormalities in external segmentation, such as lateral variation in segment size and number, are in all probability directly attributable to earlier abnormalities in the segmental arrangement of the mesoderm. However, segmentation of mesoderm and attachment of muscles to the body wall are not in themselves sufficient for the appearance of external segmentation, for embryos are found in which there is no apparent external segmentation, although muscle fibers are formed, segmentally arranged, and attached to the hypodermis (Fig. 3). Restricted growth of the hypodermis and failure of apodemal formation may be responsible for abnormalities of this type.

Abnormalities in differentiation of muscle fibers (Fig. 5) become apparent at 10 to 11 hours, that period during which differentiation of the body musculature occurs in normal embryos. In the normal embryo, muscles are formed from the fusion of single cells which then elongate into the typical body muscles. The most common abnormality in *fu* embryos is the formation of abnormal clumps of mesodermal cells which have become differentiated into identifiable muscle cells. These do not become attached to the body wall and never elongate. Small clumps of these cells sometimes are attached to the dorsal surface of muscle cells which have undergone normal differentiation. Single elongate cells may fuse only at their tips, forming fan-like strcutures or rosettes; these two types of cell groups may also be found in association with normal fibers. Single cells may elongate or remain relatively undifferentiated. Clumpe of cells are found in all *fu* embryos in which differentiation has taken place. All these abnormalities in the differentiation of the body musculature appear to represent differences in degree rather than in kind. Those muscle cells which form fibers are capable of complete differentiation as indicated by the presence of striated muscle in the oldest embryos. Since the living embryo shows active muscular movement, it is

apparent that the muscular system is functional; however, these movements may not be as coordinated or as vigorous as those of normal embryos.

Distribution and differentiation of the visceral musculature reflect the degree of normality of the development of the midgut. Regions of the midgut are sometimes found which do not have a well-developed musculature, but in these instances, part of the gut rudiment has remained in the posterior region (see below) and has affected movement of the muscle anlagen.

Fig. 5. Longitudinal section of 15 hour *fu* embryo showing abnormalities in muscle differentiation. At *a* and *c*, clumping and partial elongation are shown. The fan-shaped type of aggregation is shown at *b* while at *d* is an aggregation of cells and fibres which is transitional between fan-shape and rosette shape aggregations. 280 ×. *MUS* body musculature

Fat bodies. Separation of the fat body primordium from the mesoderm occurs at the same time as in normal embryos, and the subsequent development is normal in almost all embryos. Rarely, the fat body may be enlarged on one side, perhaps as a result of unequal distribution of the mesoderm earlier in development.

Dorsal vessel. The dorsal vessel is never present in *fu* embryos. In most embryos, the primordium of the dorsal vessel apparently never forms.

Nervous system. The early history of the nervous system in *fu* embryos is comparable to that of normal embryos except that the nervous system tends to form very much to one side, usually the same side on which the stomodaeal invagination will form. The primordia of the circumoesophageal ganglia or "brain" lie abnormally anterior to the developing stomadaeum. It is probable that they form in their "normal" position but foreshortening of the anterior end distorts spatial relationships in this area so that they appear to be abnormally situated. The dorsal primordia of the "brain" are frequently at slightly different anterior-posterior levels and are also affected by the unilateral anterior extension of the yolk, with the result that one of the supraoesophageal ganglia is frequently smaller than the other.

Yolk granules are frequently involved in the developing nervous system. If the clumps of yolk are small, they apparently can be incorporated without damage to the embryo, and isolated yolk globules are occasionally found included in the nervous system of normal embryos. When large amounts of yolk are present, abnormalities in the nervous system may result. At about 8 hours, the ventral wall of the primitive gut or yolk sac in some lethal embryos ruptures, releasing large amounts of yolk in the region of the developing nervous system. As a result, the ventral nervous system may consist of isolated regions of differentiated tissue. Escaping yolk may also spread apart the two halves of the ventral nervous system.

Fibers make their appearance in the nervous system of *fu* embryos at the same time as in normal embryos. After shortening of the embryo, it is apparent that the conformation of the nervous system is abnormal, the ventral nerve cord frequently varying in width and depth along its length. The fibers are covered in some areas by a layer of ganglion cells as in the normal embryo, while in other areas, the fibers are directly exposed to the surface. The course of the fibers may also be erratic.

Separation of the ventral nervous system from the hypodermis may fail to take place, or take place only partially (Fig. 2). Normal condensation of the nervous system never occurs, although a few embryos are found in which some has taken place.

The first signs of cellular abnormalities in the nervous system appear at gabout 11 hours. At this time large clear nuclei may be observed among the ganlion cells in the region of the ventral surface of the fibers. The ganglion cells appear to be loosely aggregated in some embryos at 16—17 hours and the fibers become necrotic later. Cellular differentiation is normal in about half of the embryos over 17 hours of age. Deterioration of the nervous system in *fu* embryos apparently does not follow a general pattern as in the case in embryos homozygous for the gene *Krüppel* (*Kr*) (Gloor 1950).

Spatial relationships of the elements of the nervous system in the anterior end are very distorted. Formation of the "brain" may be so abnormal that the oesophagus is not surrounded by the circumoesophageal ganglia at all, but runs beneath them. Failure of head involution increases the degree of abnormality in the general configuration of the nervous system. Failure of hypodermal formation may lead to the exposure of the "brain" dorsally (Fig. 6); in other regions of the embryo, the hypodermis may fail to cover the entire surface and frequently the ventral nervous system is also exposed.

Tracheal system. In normal embryos, the tracheae develop from separate pits which invaginate, elongate differentially and finally fuse (Poulson 1950). The first indication of abnormality in the tracheal system in *fu* lethal embryos is the variation in depth of tracheal invaginations shortly after they appear (7 hours). The direction of elongation of the rudiments in lethal embryos may vary and this becomes increasingly apparent as elongation continues. The undulations in the course of the two main tracheal trunks (Fig. 4) which are normally straight may be apparent as early as 10 hours. At the same time it becomes obvious that fusion is abnormal, some regions having fused while others have not. This condition of incomplete fusion persists and results in a main tracheal branch which is made up of isolated segments (cf. Fig. 1). The distortion of the

tracheal branches is more apparent in the middle segments than at either end. Both posterior and anterior spiracles are normal. The movement of the main branches from their lateral origin to the dorsal surface is frequently not completed. As a result, the main branches may run near the ventral surface for at least a part of their length. Chitinization of both main trunks and small branches may be incomplete, and segments of the tracheae are often torn and twisted. In living embryos the tracheae fill with air even when the branches are incomplete. This means that the physiological mechanisms responsible for the filling of the tracheae are operative in the lethal embryo.

Hypodermis. Differentiation of hypodermal cells in lethal embryos takes place at the same time as in normal embryos (8 hours). At this time it is evident that cells of the hypodermis which are directly beneath large clumps of free or phagocytized yolk are more flattened than other hypodermal cells. Growth of the hypodermal cells over the dorsal surface seems to be affected in many embryos, perhaps as a result of the "skewness" and in such embryos both "brain" and gut may be exposed to the surface (Fig. 6). The ventral hypodermis is also incomplete in some embryos. This may be due to secondary break-throughs or incomplete development in those regions where abnormal amounts of yolk near the surface cause structural changes in the developing hypodermis in younger embryos. Some embryos are also found in which gaps in the hypodermis are evident at the time of its differentiation. This may be related to restricted mitotic activity. Development of intersegmental folds corresponds generally to the abnormalities in segmentation which have been discussed. In some instances these folds do not form although segmental attachment of the lateral musculature appears to have taken place (Fig. 3). Differentiation of the chitinous layer and of the setae proceeds normally in time. However, spatial abnormalities do exist, the most obvious of which is the pattern of the setae in the ventral posterior region (Fig. 1).

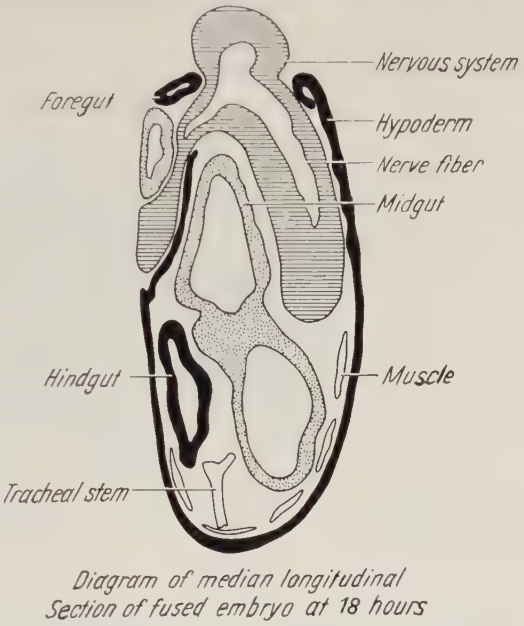

Fig. 6. Diagrammatic representation of 18 hour *fu* embryo in which the hypodermis is incomplete and the nervous system and gut are exposed to the surface

Head involution. Involution is never normal in *fu* embryos (Figs 2, 3 and 4). At best it is extremely rudimentary, resulting in a distorted and foreshortened cephalopharyngeal apparatus. In many embryos, little or no involution occurs.

Abnormalities in configuration of the head region are conspicuous features of the *fu* embryo at 5 to $5^1/_2$ hours. These result from abnormalities in morphogenetic movements described earlier. The displaced yolk causes a bulging of the

anterior tip and the developing stomodaeum is pushed toward the ventral surface. As a result of these displacements, the characteristic appearance of *fu* embryos between 6 and 12 hours is produced (Fig. 7). The anterior tip is foreshortened and blunted and frequently an odd "tuft" of cells forms at the anterior end and to one side near the dorsal surface. The ventro-lateral origins of the stomodaeum and subsequent developmental events in this region will be described in the section on gut development (see below). In all *fu* embryos, the large ventral muscles in the first thoracic segment fail to attach at the anterior end. The failure of these muscles to attach properly may be in part responsible for the failure of involution. Cells forming the frontal sac are present in the *fu* lethal

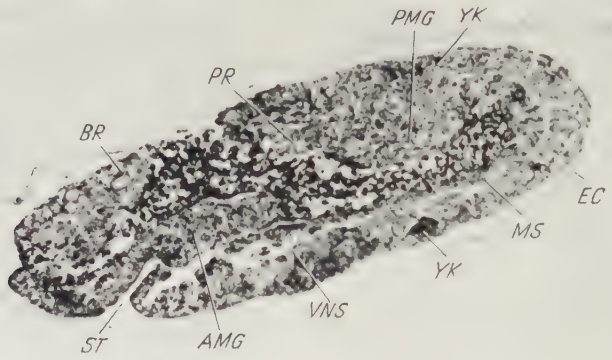

Fig. 7. Longitudinal section of 6½ hour *fu* embryo showing the abnormal shape of the anterior end. Compare with Fig. 44 in Poulson (1950). The foreshortening of the stomodaeum is also apparent. 200×. *AMG* anterior midgut; *BR* brain; *EC* ectoderm; *MS* mesoderm; *PMG* posterior midgut; *PR* proctodaeum; *ST* stomodaeum; *VNS* ventral nervous system; *YK* yolk globules

embryos and are capable of differentiation as evidenced by the production of large areas of pigmented chitin on the anterior surface. In some embryos these areas produce recognizable structures such as mouth hooks, pharyngeal bars, the median tooth and the chitinous plate of the under lip. In a few embryos, it is possible to distinguish the large lateral plates of the cephalopharyngeal apparatus. The mouth hooks usually form laterally and may point in opposite directions.

The Alimentary System. The digestive tract in the normal embryo is a compound structure, the foregut and hindgut being derived from ectoderm and the midgut from three separate rudiments which may be classified as endodermal or mesendodermal depending upon one's interpretation of germ layers in the early embryo (Poulson 1950). The salivary glands are ectodermal in origin, and the Malpighian tubules develop as out-pocketings from the posterior midgut primordium near its junction with the proctodaeum (Poulson 1950).

The stomodael invagination in *fu* embryos is abnormal from the time it becomes apparent. It is not formed in the ventral anterior midline as in the normal embryo, but invaginates laterally almost directly under the anterior tip of the embryo and is abnormally wide and shallow. Its actual formation may be delayed, and after it forms, cytoplasm frequently sloughs off into the cavity of the invagination in early stages of its formation. It never attains the length of the stomodaeum in normal embryos. There is probably a genuine shortening

of the invagination but this is exaggerated by a tendency of the stomodaeum to grow toward the midline. There appears to be decreased mitotic activity, but this is based on a general impression rather than on definite cell counts. At first the angle of the invagination is flattened as a result of abnormal spatial relationships but later the angle appears to be somewhat steeper than in normal embryos. Cells of the stomodaeum seem capable of normal differentiation, for at eight hours, the invaginationa of the primordia of the stomodaeal nervous system and of the corpora cardiaca form normally (Fig. 8). At this same time, it is obvious that contact between the anterior midgut rudiment and the stomodaeum is abnormal in some embryos; in the most extreme cases, no contact is

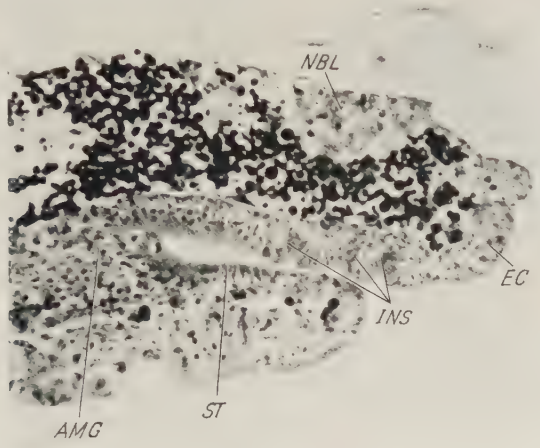

Fig. 8. Longitudinal section of anterior end of 8 hour *fu* embryo showing the invaginations which give rise to the stomodaeal nervous system and the corpora cardiaca. Compare with Fig. 14 in POULSON (1950). Note also the foreshortening of the lower "jaw" of the abnormal embryo. 400 ×. AMG anterior midgut; *EC* ectoderm; *INS* invaginations in stomodaeal roof; *NBL* neuroblast; *ST* stomodaeum

apparent between the two. This is also evident at a later stage in development when it is obvious that the lumen of the foregut is not confluent with that of the anterior midgut. This results in proventricular abnormalities which will be discussed below. Regions of the foregut which do not move to the interior of the embryo as a result of faulty head involution take on the characteristic appearance of the pharyngeal floor (Fig. 4). It is frequently possible to determine the degree to which movement of these regions to the interior has taken place in abnormal embryos by the position of these differentiated areas. Larval differentiation of the foregut occurs as in normal embryos, except for differentiation of certain proventricular structures discussed below.

In normal embryos, the oesophagus extends into a vase-like portion of the midgut, reflects back on itself and then joins the midgut, forming the structure commonly known as the proventriculus. The outer layer of this extension develops into a layer of large clear cells which stain lightly. These cells are differentiated in the normal embryo shortly before hatching and apparently act as a "press" in the formation of the peritrophic membrane which cells of the gastric portion secrete (WIGGLESWORTH 1950). Structural faults in the proventriculus of late *fu* embryos are related to three events earlier in development: 1. the degree

to which spatial relationships between the stomodaeum and the anterior midgut rudiment are abnormal; 2. foreshortening of the gastric portion as a result of abnormalities in the midgut rudiment; and 3. shortening of the foregut. The fai ure of head involution may also be an important factor. In 15 out of 51 "mlature" embryos, the proventriculus was normal except that the large clear cells did not form (Fig. 2). In the remaining embryos, abnormal union between the gastric and oesophageal portions resulted in aberrant proventricular structure (Fig. 4). In all *fu* embryos at this stage, formation of the peritrophic membrane is abnormal, resulting in the deposition in the gastric portion of a large mass of chitin which becomes pigmented at the same time as do other chitinous structures in the embryo. This abnormality results from the failure of the large clear layer of cells to form and act as a "press" in the shaping of the peritrophic membrane.

The rudiments of the midgut form at the same time as in normal embryos, but the asymmetry of the anterior and posterior midgut rudiments is apparent at six hours. The anterior midgut is usually toward the side of the embryo on which the stomodaeum forms, and the posterior midgut and proctodaeum on the other. The lumen of the anterior midgut remains open to the surface for a longer time than in normal embryos. As the anterior midgut grows posteriorly, there is frequently differential growth in the two arms of the rudiment, the arm on the side of the stomodaeal invagination tending to extend more to the rear. Although growth and fusion of the midgut rudiments usually occur in abnormal embryos, they may not occur normally which results in aberrant gut formation and differentiation. In some embryos, it is apparent that not all of the cells of the posterior rudiment move anteriorly as in normal embryos, but remain in clumps at the posterior end. Frequently cells of the middle midgut rudiment are also incorporated in this posterior region instead of migrating to the anteriorly growing arms. There is apparently a tendency for both anterior and posterior rudiments to grow toward the midline.

These early abnormalities express themselves in aberrant development and differentiation of the larval gut. In only about a third of the embryos which have undergone larval differentiation does the gut approximate that of a normal embryo (Fig. 4). Even in these, the gastric caecae are rudimentary or absent, and in some the gastric portion of the proventriculus is foreshortened, making it apparent that in all embryos the anterior midgut rudiment and its derivatives are to some extent affected. In a few embryos, coiling of the gut and cellular differentiation, processes which bear a close relationship to each other, are retarded and abnormal. In more than half of the embryos in this age group, there is failure of complete constriction; the gut remains sac-like dorsally but is partially coiled ventrally (Fig. 2). The gut wall in such embryos varies in thickness, with large clusters of cells apparent in some regions, although the gut is normally a single layer in thickness. These clusters of cells are most prevalent in the region of the posterior midgut. It is probable that the formation of a complete gut in spite of the failure of some of the primordial cells to take part in the process, is related to the power of regulation in the embryonic gut. It is apparent that when cells of the gut for some reason do not move to their definitive positions and remain clumped together, other cells of the gut become abnormally thin and elongated.

This may result in an extremely fragile gut wall and it is not uncommon in *fu* embryos to find the gut wall broken and yolk escaping into the haemocoel. Rarely, the gut does not undergo differentiation and retains the sac-like structure characteristic of the 12 hour normal embryo. In all *fu* embryos, large amounts of yolk are present in the gut at a late stage of development (Figs. 2 and 3) although there is little or none remaining in the gut of the normal embryo at this time. This may be indicative of impaired powers of yolk utilization.

The hindgut may develop slightly to one side as a result of displacement of the primordium toward the end of gastrulation. In some embryos, the shortening of the embryo is apparently not complete, and the anal opening in these embryos, although at the posterior tip, is nearer the dorsal surface than in normal embryos. Part of the surface ectoderm which normally forms a part of the hindgut frequently fails to be carried into the interior at this time (9—10 hours) and remains as a thickened hypodermal region at the posterior tip. These ectodermal "lips" are so large that they may be obvious in living embryos (Fig. 9). Larval differentiation is usually normal.

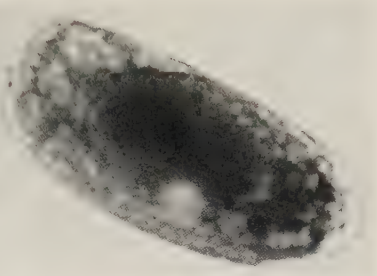

Fig. 9. Still from film of developing abnormal embryo about 14 hours of age. The incomplete involution of the hindgut has resulted in the formation of conspicuous ectodermal lips at the posterior tip. The abnormal shape of the anterior end is also apparent in the living embryo. 100×

The salivary glands are frequently asymmetrical in arrangement, one being pulled nearer the midline and more anterior than is normal. This asymmetry is frequently evident at the time of formation of the salivary gland plates. In some instances, the ducts of the gland fail to move to the interior as a result of abnormal head involution and remain open to the ventral surface. In other respects the salivary glands are apparently quite normal. After 19 hours, signs of degeneration appear in the glands of a few embryos. The glands become distended over all or part of their length and the cells are thin and flattened. At the same time the nuclei of the cells become pycnotic.

The degree of abnormality in development of the Malpighian tubules is closely associated with the degree of abnormality in midgut differentiation. When development of the gut is normal or nearly so, the Malpighian tubules are apparently normal. In embryos in which the midgut is grossly abnormal, the Malpighian tubules are rudimentary or absent.

Gonads. The gonad sheath, which is of mesodermal origin, is normal in *fu* embryos. The two gonads are not located in the same anterior-posterior positions in the body cavity as they are in normal embryos, one usually being nearer the anterior end. They may also occupy different dorsal-ventral positions. Although the germ cells appear normal and undergo characteristic mitoses at about sixteen hours, germ cells show signs of deterioration in some embryos between the ages of 19 and 24 hours. The cytoplasm of the cells becomes vacuolate and stains darkly while the nuclei show evidence of pycnosis.

The pattern of damage in embryos produced by matings of mutant females to non-mutant males

Forty-eight embryos between the ages of 17 and 24 hours from crosses of *fu* females to *Ore K* males were sectioned. Twenty-eight of these were classified as males, sixteen as females and four as probable females on the basis of gonad size and the relationship of the gonad to the fat body.

In general the pattern of damage in the lethal *fu*/*Y* embryo does not differ in any major respects from that of lethal embryos from mutant matings. However, the general tendency seems to be toward a less extreme manifestation of the effects of the gene.

In three of the male embryos, complete differentiation of the large clear cells of the proventriculus had taken place although this was never found in matings where the parents were both of the mutant genotype. In sixteen of the embryos, the proventriculus was normal except for the differentiation of these cells. Abnormalities in the manner of junction were present in the remaining embryos and did not differ from those observed in matings of mutant to mutant.

Table 2.
Incidence of abnormal abdominal segmentation in adult females from crosses of fu and fu^{ll} females to Ore K males, and in the cross of Ore K females to fu males

Female Genotype	Parents		Total Adult Females	Abnormal Abd. Seg.	Percent Abnormal
	Female	Male			
fu/+	*fu*/*fu*	+/*Y*	332	224	67
fu^{ll}/+	*fu^{ll}*/*fu^{ll}*	+/*Y*	247	200	80
fu/+	+/+	*fu*/*Y*	160	5	3

Differentiation of the body musculature was more nearly normal in male embryos when the father was non-*fu*. Fibres were better developed and the only type of abnormal differentiation which was found was the formation of clumps of muscle cells.

In general, development of the midgut was more nearly normal in lethal embryos when the father was non-*fu*, and considerably less yolk was present in the gut. Malpighian tubule development again reflected the degree of normalcy of gut development.

Abnormalities in segmentation, bristle differentiation and distribution, and head involution did not differ between lethal embryos of mutant and non-mutant fathers. The dorsal vessel was also absent in all embryos.

Four embryos which were classified as females because of size and position of the gonad were found to be abnormal in many respects. In all four, involution of the head was incomplete, segmentation was irregular, the proventriculus was abnormal and the dorsal vessel was absent or highly abnormal. It is possible that the small number of germ cells in the gonad is not an infallible method of identifying female embryos, especially when development is abnormal. However, because the number of females which emerge in crosses of mutant females to non-mutant males is low and because there is a maternal effect of the *fu* gene on females which do emerge (see below), it does not seem unlikely that in some female embryos from such crosses development would be abnormal.

Sixteen of the embryos which were classified as females on the basis of gonad size were found to be normal in every respect except for abnormalitis in differentiation of the body musculature. Clumps of muscle cells were frequently found

similar to those observed in lethal embryos. Associated with this abnormal differentiation in the embryo was a high incidence of abnormal abdominal segmentation in adult heterozygote females when the mother was of the mutant genotype (Table 2).

Embryonic patterns of damage associated with the alleles fu^g and fu^{ff}

The final pattern of damage was studied in detail in 21 fu^{ff} embryos and 34 fu^g embryos, between the ages of 18 and 24 hours (Table 1, columns 3 and 4). A thorough analysis of the development of embryos from fertilization to larval differentiation was not carried out.

An examination of Table 1 will show that the general pattern of damage associated with these alleles is the same as that for *fu*. Involution of the head fails or is partially completed and there is a foreshortening and distortion of the cephalopharyngeal apparatus. The course of the oesophagus and structure of the proventriculus are abnormal. The dorsal vessel does not form, and there is a general asymmetry in the arrangement of various organs.

Certain differences in the pattern of damage are indicated however. Abnormal segmentation and concomitant abnormalities in bristle distribution are less marked in fu^{ff} embryos and the degree to which involution of the head takes place is more consistent. However, cellular degeneration of germ cells and salivary gland cells occurs in these embryos much earlier and with a greater frequency than in the other two groups of embryos, and there are also abnormalities in the formation and differentiation of the posterior tracheal trunks. Differentiation of the midgut is usually normal in both fu^{ff} and fu^g embryos, and most of the yolk in the gut is utilized. Development of the Malpighian tubules is normal in fu^{ff} embryos, but is rarely normal in fu^g embryos.

Discussion

Although the developmental consequences of the action of *fu* are relatively easy to follow in time and space, the fundamental action of the gene remains obscure. At about 5 to $5^1/_2$ hours, the *fu* embryo suddenly becomes abnormal. The changes are rapid and as yet no intermediate stages between normal and abnormal embryos have been found. Films made of the abnormal embryo during the course of development simply show that the anterior-posterior extension of the embryo at the end of gastrulation does not occur in the same way as in normal embryos. While it seems probable that growth must be retarded or restricted differentially because of the unilaterality of the abnormal extension it has not been possible to demonstrate this. Certainly it is not associated with visible cytological abnormalities.

Abnormalities appearing later also point to restrictions in growth. Some examples we may cite are: incomplete formation of the ventral hypoderm; the shortened stomadaeum; foreshortening of the gastric portion of the proventriculus and absence of gastric caecae (abnormalities of the anterior midgut rudiment?); failure of head involution; abnormal elongation and fusion of tracheal branches; and partial constriction of the midgut. It is not possible to determine whether these abnormalities are caused by a generalized inhibition of growth over a prolonged period or whether they are result of a single event early in

embryonic life. The difficulties are compounded because, in many instances, the factors involved in these processes in normal development are not yet clear.

It is evident that many of the characteristics of the final pattern of damage are related to the action of the gene only in they are related to spatial distortions established early in development in response to the mutant gene.

Many of the effects of the gene, indeed the lethality of the gene itself, can be overcome by the presence in the zygote nucleus of a normal X chromosome. On the other hand, certain defects in development persist even when the zygote nucleus contains the normal allele. One of these defects concerns the structure and composition of the oöcyte and will be dealt with in detail in a later paper. Another persistent defect which is associated with the genotype of the mother is the abnormal segmentation observed in all lethal embryos and in heterozygous females when the mother is of the genotype. Abnormal preblastodermal localizations in the unfertilized egg which the redemptive effect of the normal gene cannot overcome may be responsible for this abnormal situation.

Although the pattern of damage in lethal embryos *(fu/Y)* in crosses where the father is non-*fu* is very like that in lethal embryos where both parents are mutants, it tends to be less severe in some respects. In male embryos from both crosses, the composition of the egg cytoplasm, the zygote nucleus and the polar body nuclei are identical (see Fig. 1, Counce 1956). The only apparent difference is in the composition of the chromosomal make-up of the supernumerary sperm, some of which in all probability contain the normal X chromosome when the father is non-*fu*. This may mean that the differences observed in the degree of severity in the pattern of damage in male lethal embryos in the two crosses is related to some active role of supernumerary sperm in development. Some indications of such a role have been presented for *dor* (Counce 1955) and further indications will be discussed in connection with *r*.

Although Poulson (1940, 1945) and Bull (1952) have studied the effects of overlapping deficiencies on embryonic developemnt in *Drosophila*, no comparable studies of allelic lethal genes have been made. As three alleles at the *fu* locus were available, the final pattern of damage associated with each of the alleles was studied (Table 1). Although there are obviously differences in the patterns of damage, it would be impossible to classify any single embryo as being of a specific genotype on the basis of the pattern of damage. This difficulty is intensified by the variations in manifestation of the pattern of damage within any one genotype. Any number of factors may influence the manifestation of a gene and even the phase specificity itself of lethal action can be modified. Hadorn (1951) found that the time of lethal action of *Curly* homozygotes could be influenced by temperature and genetic milieu; the age of the female also was an important factor (Hadorn and Zeller 1943). Even after the *Curly* lines were made isogenic, variations were found which Hadorn (1951) attributed to " . . . the variability that is presumably inherent to any developing system". Hadorn (1951), by means of selection, was able to separate two genotypes of the *lethal giant larva* stock which differed with respect to time of lethal action; in one line, alsmost all the homozygotes died in the late embryonic stage, while in the other line, they died during the third larval instar. The lethal effect of the mutant *cryptocephal* (Hadorn and Gloor 1943; Gloor 1945) has been found to be

affected by genotypic milieu, sex of the individual and breeding temperature. Mutants are known in *melanogaster* and other *Drosophila* species in which lethality is expressed only at certain temperatures (Spencer 1942; Dobzhansky 1946; Meyer et al. 1949). One must take all these factors into consideration if a valid comparison of allelic systems of lethal genes is to be made, and this has not been done in the present study. However, the variations in extrinsic factors such as temperature, age of females, etc., is as great within lines as between lines. None of the genes was carried in an isogenic line so that there were genotypic variations within lines as well as between lines. There seems to be an indication at least that there are allelic differences in the pattern of damage associated with lethal genes, just as there are allelic differences in visible phenes.

Another interesting facet of the pattern of damage in *fu* embryos is the close resemblance it bears to the patterns of damage associated with other lethal factors (Table 1). The patterns of damage of *Krüppel (Kr)* (Gloor 1950) and *Minute IV deficiency (M-4)* (Farnsworth 1951a, b) are strikingly similar. These in turn have, in common with other lethal factors, certain characteristics which seem to form a syndrome. These characteristics involve abnormal head involution and abnormal segmentation, frequently accompanied by degeneration of the nervous system after it differentiates in a normal fashion. Bull (1952) has described this syndrome as associated with certain lethal *vestigial* deficiencies. Ede (1954) studied abnormal embryogenesis in a number of sex-linked lethals. Of these, four — *Lff* 11, *X*-2, *X*-27 and *X*-10, type III — showed this typical pattern of abnormality. Ultrasonic treatment at the syncytial blastoderm stage (Selman and Counce 1953; Counce and Selman 1955) frequently produces abnormal differentiation of this type. There seems to be little relation to the time at which development becomes visibly abnormal and this syndrome of effects. Bull (1952) reported that the first signs of abnormality in *vestigial B* are associated with mitoses and can be traced back to early cleavage stages while both Gloor (1949) and Farnsworth (1951a, b) were unable to detect differences in the normal and abnormal embryos prior to the time of segmentation. *Fused* has its first visible effect at the time of gastrulation, while one of the lethals described by Ede first becomes abnormal at the time of blastoderm formation, and another at the time of formation of the cephalic furrow. Apparently both involution and segmentation are processes which are especially vulnerable to any adverse chenges which may have occurred at previous times during development. They are "epigenetic crises", in the sense of Waddington (1941); in his worde, "general alterations of conditions are as it were stored up until then, and precipitated at the time into specific modifications". It is also interesting to note that *Kr* in the heterozygous state affects the structure of the thorax, probably related to abnormal segmental structure earlier in development, while *fu* is able to cause abnormal abdominal segmentation if the egg is formed in a female of the *fu* genotype. Deterioration of the nervous system follows regular patterns in both *Kr* (Gloor 1950) and *M*-4 lethals (Farnsworth 1951a, b) but this is apparently not the case with *fu*. Poulson (personal communication) has suggested that abnormal innervation and/or tracheation may play an important role in the degeneration of the nervous system in these lethals.

A general discussion of the effects of *fu* as compared with those of *dor* and *r* will be presented in a later publication as will the general implications of mutants of this type.

Summary

1. The effect of the female sterility gene *fu* on the development of zygotes is described.

2. In matings of *fu* females to *fu* males, abnormalities in development become apparent at the end of gastrulation. These result in the establishment of a general asymmetry in the germ layers which is never overcome and which is responsible for many of the ensuing abnormalities in development. Eventually an embryo is produced which resembles in many respects a fully differentiated larva, but which shows certain abnormalities which form the pattern of damage. No embryos emerge from the embryonic membranes.

3. The pattern of damage in male embryos from crosses of *fu* females to non-*fu* males is similar to that observed in crosses of mutant to mutant. Female (non-lethal) embryos in such crosses exhibit abnormalities in differentiation of the segmental musculature. A^c'ult heterozygous females from such crosses show a high incidence of abnormal abdominal segmentation, although the heterozygous female does not when the mother is not of the mutant genotype.

4. The patterns of damage in two alleles of the *fu* gene — fu^g and fu^{ff} — are generally the same as the pattern in *fu*, although there are indications of allelic differences in the manifestation of gene action.

5. A primary effect of the gene *fu* on growth processes is postulated.

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different from that of r^{41} females. Every female of the r^{41} genotype in this experiment produced some eggs although the number produced by individuals varied from a maximum of 73 eggs on one day by one female to the production of no eggs by two of the females on one of the three days.

In another experiment, the frequency of malformed and unfertilized eggs was recorded. The results are given below in tabular form.

The differences among all three genotypes as regards numbers of unfertilized and abnormal eggs were significant (X^2 is 31.4 with 2 degrees of freedom; $P < 0.01$).

The high proportion of abnormal and unfertilized eggs in r^H females, combined with low egg production, made it difficult to get enough embryos for a study of the effect of the gene on embryonic development, and most of the embryological work was done with r^{39} and r^{41} embryos.

Table 2. *Number of unfertilized and abnormal eggs produced by r^H, r^{39} and r^{41} females****

Genotype	Number of eggs examined	Unfertilized eggs, normal shape	Unfertilized eggs, abnormal shape*
r^H	163	59 (36.0%)	57 (35.5%)
r^{39}	200	39 (19.5%)	36 (18.0%)
r^{41}	190	22 (11.5%)	25 (13.0%)

* Such eggs were much smaller than normal eggs and were extremely opaque. Frequently they collapsed

** Matings of mutant females to *Ore K* males

The pattern of damage in embryos

Although most *r* lethal zygotes die before hatching, a few emerge from the embryonic membranes. All such larvae are extremely sluggish, soon become flaccid, and die without moving far from the egg membranes from which they have emerged. Since ". . . the lethality crisis becomes manifest at the boundary between the end of embryonic (*E*) and the beginning of larval (*L*) life", Hadorn would classify this as an *E*/*L* boundary lethal (1951). However, the events which lead to the death of the animal have occurred during embryogenesis.

At the time of hatching, living embryos appear very much like normal embryos except that the tracheal branches rarely fill with air, and in some embryos, abnormalities in segmentation are apparent. The movements of lethal embryos are not as vigorous as those of normal embryos of the same age.

Development of *r* embryos is apparently normal up to the period of cellular differentiation which occurs between the 13th and 16th hours of development. In other words, early development, gastrulation, histogenesis and primary organogenesis follow the same patterns as in normal embryos. Those processes of cellular differentiation which take place between 13 and 16 hours in normal embryos rarely if ever occur in *r* embryos, and processes of differentiation which normally occur after 16 hours are delayed.

The embryos on which the following discussion is based consisted of 24 r^{39} embryos and 35 r^{41} embryos between the ages of 20 and 24 hours. In addition 16 r^9 embryos 25 or more hours of age were studied. Onyl 4 r^H embryos in the 20–24 hours group were available for study and therefore no certain conclusions may be drawn concerning the effect of this gene on embryonic development. However, these four embryos and several which were younger did not differ in any major respects from other *r* embryos and we may assume that the effect of r^H on development is very like that of r^{39} and r^{41}. Many embryos of all three genotypes less than 20 hours of age were observed.

The effects of the genes r^{39} and r^{41} as observed in embryos between 20 and 24 hours of age are presented in tabular form in Table 3. Because there is an apparent difference between embryos as regards differentiation at 20–$22^1/_2$ and $22^1/_2$–24 hours of age, the general appearance of embryos at these two periods is presented separately.

Differentiation of body musculature is affected in embryos of all four genotypes. In all but a few embryos, clumps of undifferentiated muscle cells are found, especially in the posterior regions. Although cellular differentiation of the body musculature begins between 11 and 12 hours, it is not completed until about 14 hours (POULSON 1950).

Differentiation of the fat bodies is retarded, but does take place and in the $22^1/_2$ hour embryo, differentiation appears normal. Degeneration begins soon after, and in r^9 embryos, all of which were 25 or more hours of age, degeneration of the fat bodies was advanced.

The dorsal vessel forms in all *r* embryos but the retardation in development is marked. In r^{39} embryos, the dorsal vessel in a 20 hour embryo resembles that of a normal embryo between 14 and 16 hours of age. In r^9 embryos, the dorsal vessel had almost completely deteriorated.

Development of foregut and proventriculus is approximately the same in all *r* embryos. In all *r* embryos, the midgut fails to develop the cellular characteristics of the larval stage. In addition, development of the gut is abnormal in about one third of all r^{39} and r^{41} embryos, and over half of the r^9 embryos. These abnormalities in gut formation are largely the result of abnormal cell migration much like that observed in *fu* embryos (COUNCE 1956b). The midgut remains packed with yolk, little or none being utilized.

Malpighian tubules are rarely normal, which is to be expected since the development and differentiation of the tubules and the midgut seem closely related (COUNCE 1956b). In older embryos, the tubules become quite swollen, perhaps as the result of accumulation of excretory products.

Larval differentiation of the hindgut rarely takes place. In a few embryos there is some differentiation after $22^1/_2$ hours, but this is usually restricted to a short region of the hindgut extending from the anus to about the region of the anterior end of the seventh abdominal segment. The transition to the wider lumen of the undifferentiated hindgut is abrupt, and gives the appearance of a constriction in the region. There is no apparent reason for this partial differentiation, but the differentiated portion may represent the region of the hindgut which is formed by cells from the surface which move to the interior at the time of shortening (COUNCE 1956b).

In some of the oldest embryos, the nuclei of the cells of the salivary glands take on an appearance like those of the glands of the first instar larvae, indicating that their powers of further differentiation are not impaired. Other examples in which stages of differentiation characteristic of late embryos or early first instar larvae occur in *r* embryos after $22^1/_2$ hours are the appearance of muscle striations and the differentiation of the cells of the proventricular region of the oesophagus which act as a "press" in the formation of the peritrophic membrane.

The morphology of the nervous system is apparently normal. This is to be expected since it differentiates before the period when the aberrant influences come into play. However, there seems to be an effect at the physiological level

Table 3. *The patterns of damage as seen in r^{39} and r^{41} lethal embryos between the ages of 20 and 24 hours*

Genes	r^{39}		r^{41}	
	20–22½ hours	22½–24 hours	20–22½ hours	22½–24 hours
Body musculature	Differentiation usually abnormal, especially posterior regions.	- -	As r^{39}	—
Visceral musculature	Affected only in embryos with highly abnormal gut differentiation.	- -	As r^{39}	—
Fat bodies	Differentiation retarded.	Differentiation normal by 22½ hrs. May begin to deteriorate by 24 hrs.	Differentiation retarded, less marked than in r^{39}.	Differentiation normal by 22½ hrs. May begin to deteriorate by 24 hrs.
Dorsal vessel	Marked retardation in development and differentiation.	Larval differentiation. Nuclei become pycnotic by 24 hrs.	Differentiation retarded.	Larval differentiation. Nuclei become pycnotic by 24 hrs.
Nervous system	Normal differentiation, but condensation may be retarded.	Necrosis of nervous system in all embryos.	Normal differentiation.	Necrosis of nervous system in over 50% of embryos.
Tracheal system	Abnormal in all embryos. Lumen and posterior spiracular chamber narrow. Posterior spiracles rudimentary. Tracheae fail to fill with air.	Cells in posterior region show signs of degeneration by 24 hrs.	As r^{39}	As r^{39}
Hypodermis	No larval differentiation of hypodermis in posterior regions in 50% of embryos. Bristle differentiation normal in anterior regions.	—	No larval ifferentiation of hypodermis in posterior region in 12% of embryos. Few or no bristles formed in 15% of embryos.	—
Head involution; mouth parts and cephalopharyngeal apparatus.	Head involution normal in most. Pigmentation of chitinous structures delayed.	Full pigmentation of chitinous structures.	As r^{39}	As r^{39}
Segmentation	Usually abnormal, weakly expressed posteriorly.	-	As r^{39}	As r^{39}
Foregut	Larval differentiation retarded in about 50% of embryos.	Most embryos show normal differentiation.	Most embryos show normal differentiation.	—
Proventriculus	Differentiation of oesophageal portion retarded. Gastric portion abnormal in 15% of embryos, resulting in abnormal union.	Larval differentiation.	As r^{39} except incidence of abnormal union 33%.	Larval differentiation.

Table 3 (Continuation)

Genes	r^{39}		r^{41}	
	20—22½ hours	22½—24 hours	20—22½ hours	22½—24 hours
Midgut	No differentiation. Development abnormal in about 30% of embryos—incomplete formation of constrictions, failure of cell migration.	—	As r^{39} except that gut constrictions are formed or more less completely.	—
Hindgut	No differentiation	May be differentiated in restricted area extending from anus to 7th abdominal segment.	As r^{39}	As r^{39}
Salivary glands	Secretory activity prolonged	Larval differentiation—may take on appearance comparable to first instar larva in oldest embryos.	As r^{39}	As r^{39}
Malpighian tubules	Differentiation delayed. Normal development in only 25% of embryos.	Tubules swollen, nuclei of cells pycnotic.	As r^{39}	As r^{39}
Gonads	Delay in mitotic division	Mitotic division has occurred. Germ cells in some older embryos show signs of deterioration.	As r^{39}, rarely gonad absent	As r^{39}
Yolk	Little or none utilized; gut packed full.	—	As r^{39}	As r^{39}

* In normal embryos, there is a mitotic division of the germ cells in the gonad at about 16 hours.

as necrosis of the nervous system occurs in all r^{39} embryos between $22^1/_2$ and 24 hours of development, and in over half of the r^{41} embryos in the same period. The nervous system was necrotic in all r^9 embryos and in 3 out of 4 r^H embryos, all of which were $22^1/_2$ hours of age or older. The presence of large numbers of yolk granules in the nervous system of many r embryos may contribute to the degeneration, although yolk granules have been observed in the nervous system of normal embryos where apparently have no deleterious effects. The delay in condensation in r^{39} embryos may be a contributing factor to degeneration of the nervous system since necrosis of the nervous system occurs in several lethal genotypes in which spatial relationships of the nervous system have been disturbed (Gloor 1950; Farnsworth 1951; Bull 1952; Counce 1956b).

In none of the r embryos examined was development of the tracheal system normal. Development is normal until fusion of the branches and formation of the posterior spiracles is completed. Movement of the main trunks to their definitive position near the dorsal surface is usually accomplished but in about a quarter of the embryos it too is affected, as evidenced by the rather twisted course of the main trunks. As a result of cessation of differentiation, the main

trunks are much narrower than in normal embryos, and the posterior spiracular chamber is exceedingly narrow. The posterior spiracles are also affected, their characteristic chitinous structures being only partially formed. It is possible that there is a relationship between the failure of differentiation and failure of tracheal branches to fill with air. Cells of the posterior region show signs of degeneration by 24 hours.

Involution of the head is usually normal in *r* embryos. Pigmentation of the mouth parts and chitinous portions of the cephalopharyngeal apparatus are delayed considerably (approximately 4 hours) but by $22^1{}_2$ hours, all chitinous structures in the head region are fully pigmented.

Abnormal segmentation is probably the result of abnormal muscular differentiation. Anterior extension of the segments following involution of the head does not always occur to the extent that it does in normal embryos.

Gonad formation takes place in almost all *r* embryos, although in a few r^{41} embryos, no gonad was identified. In many cases the gonads evidence the same general delay in differentiation shown by other organs. In normal embryos, there is a mitotic division of the germ cells at about 16 hours of development (Sonnenblick, 1950). This division rarely occurs in *r* embryos before $22^1{}_2$ hours, but it takes place in all *r* embryos. Signs of cellular degeneration—pycnotic nuclei and vacuolar cytoplasm—appear in the germ cells at about 24 hours. Degeneration was advanced in all r^9 embryos.

It is interesting to note that the effects on embryogenesis of r^{41} are of the same kind, but generally less severe than those of r^{39}. This is true also of other aspects of gene action such as fecundity, fertility, and percentage of abnormal eggs, which were found to differ according to genotype.

Discussion

The lethal action of r in embryogenesis

The lethal effect of *r* seems to be related to a general disturbance of processes of cellular differentiation during a limited time (13–16 hours) rather than a primary effect on a tissue or group of tissues with secondary effects on other developmental processes. In the sense that only those tissues which undergo cellular differentiation during the period affected by the action of the gene are primarily involved, the gene does have a specific effect on a group of tissues.

The result of this generalized disturbance is a retardation or cessation of larval cellular differentiation. Examples or organs or tissues in which normal larval differentiation occurs, but at a later time than in normal embryos, are the salivary glands (in which the secretory activity continues longer than in normal embryos), the mitotic division of the germ cells (probably the only mitotic activity which takes place in normal embryos after 12 hours—Sonnenblick 1950) and the pigmentation of chitin. Partial differentiation of the oesophagus, hindgut and hypodermis occurs, while cellular differentiation characteristic of the larval midgut and tracheae never takes place.

The occurrence of some embryos in which the effect of the gene seems to have begun to act earlier (incomplete involution) and others in which the action of the gene is apparently less severe, may indicate that the system affected by the action of the gene is capable of some activity but at such a low level that a required threshold value is not reached or maintained.

The possible role of supernumerary sperm in embryogenesis

An interesting aspect of the action of *r*, and one which is not evidenced by *dor* nor *fu*, is the fact that in crosses of mutant females to non-mutant males, a few males do develop into functional adult flies. This is in spite of the fact that the zygote nucleus does not contain a normal X-chromosome. LYNCH (1919) felt that the rare appearance of sons merely indicated that the action of the gene was less severe than that of *fu*. It is true that *r* has its first *apparent* effect on embryonic development at a much later stage than *fu*, and *r* embryos certainly are not as grotesque as *fu* embryos. However, *r* embryos actually succumb at about the same time or earlier than *fu* embryos (25–30 hours). Since male embryos in crosses of mutant females to non-mutant males develop from eggs produced in the ovary of an *r* female, the composition of egg cytoplasm and of the polar body complement will be identical to that of the male embryo in crosses of mutant to mutant (cf. Fig. 1a and b, COUNCE 1956a). The only difference between males in the two crosses is that sperm is furnished by a non-*r* male in one instance and an *r* male in the other. Other studies (LYNCH 1919) have shown, and it has been confirmed on the course of this work, that mutant males are completely fertile when mated to non-mutant females, indicating that their sperm are fully normal. However, since polyspermy is the general rule in *Drosophila* (SONNENBLICK 1950), the zygotes which result from fertilization by normal males must have in the egg cytoplasm some sperm containing the normal X-chromosome, and it seems possible that these supernumerary sperm may play a part in early development. The extremely low frequency of males appearing in crosses of mutant females to non-mutant males may indicate that a threshold phenomenon is involved and the lethal effects of the gene are overcome only when a certain percentage of the supernumerary sperm carry the normal X-chromosome. There are also indications from the patterns of damage in *dor* embryos and *fu* embryos from crosses of mutant females to non-mutant males that the lethal effects seem to be modified in the direction of less extreme expression (COUNCE 1956a, b). GLASS and PLAINE (1950) have cited evidence for the action of a gene at the moment of sperm entry and it does not seem improbable that at that moment the future male pronucleus has not as yet been determined.

In recent years it has been claimed by a number of Russian workers (see review by KUSHNER 1954) that supernumerary sperm may enter the cytoplasm of mammalian eggs and in some cases exert a favourable influence on the character of the offspring. These claims do not appear to have been confirmed in Western countries, and evidence which has accumulated in experiments in Edinburgh (which were, however, not specifically designed to test the point) seems to be rather against the suggestion (BEATTY, personal communication). Nevertheless the evidence described above suggests that processes of this kind may occur in certain cases.

One of the most perplexing problems in genetics today is that of penetrance and expressivity. If the supernumerary sperm do play a role in development, perhaps differences in amount or kind of certain genic substances in these sperm may play an important part in these phenomena.

A comparison of the effects of dor, fu and r on embryogenesis

Each of these three female sterility genes affects embryonic development at a different time. *Deep orange* may actually have an effect before the fusion of gamete nuclei (Counce 1956a), while *r* does not have any appreciable effect on development until larval differentiation is under way. The action of *fu* (Counce 1956b) first becomes apparent at the end of gastrulation, by which time signs of disintegration have already appeared in *dor* embryos and long before any deviation from normal development is obvious in *r* embryos. Development of *fu* embryos continues until an embryo which has many larval characteristics is produced. These embryos, which are often grotesque monsters, frequently remain alive long after the time at which normal embryos emerge from the embryonic membranes, and may live as long or longer as *r* embryos.

Although the times at which the effects of the three genes on development appear, and the ways in which the effects are manifested, are very different, there is some indication of similarity in the underlying causes. Both *fu* and *r* seem to have generalized effects; that is, each seems to cause some over-all process to go awry. *Fused* affects adversely certain morphogenetic movements, probably through a restriction or slowing down of growth rates, perhaps by an inhibitory effect on mitotic rate. *Rudimentary* causes the complete cessation of all differentiation of organs and tissues during a restricted period of time, those processes which occur before and after this period being little affected. It is not possible to speak of a generalized effect of *dor* because its action becomes obvious so early in development. At the time *dor* has its initial apparent effect, only one of two things could be visibly affected—the processes of maturation and fertilization or the cytoplasm and deutoplasm. In the case of *dor* it is most probable that the effect is expressed initially on the cytoplasm with secondary effects in the processes of maturation and fertilization.

In each of the three genes, there is an indication of the possible active participation of supernumerary sperm in development (see above).

In addition to the effects on the pattern of damage in lethal male embryos which have been observed in matings of mutant females to non-mutant males, some abnormalities in development have been noted in the females in crosses involving *dor* and *fu* females (such crosses were not studied embryologically in *r*). In *dor*, presumptive germ cells deteriorate before migration from the posterior midgut invagination. This is of interest because of the reduction of the ovary in homozygous *dor* females (and in many other female-sterile mutants as well—Beatty 1949) and the reported lower fecundity of some heterozygous females (Lynch 1919). In *fu* the maternal effect is expressed through abnormal muscle differentiation in the female heterozygote embryo, and through abnormal abdominal segmentation in the adult heterozygote.

In addition to those effects which have been mentioned, each of the three genes also affects in a characteristic manner, which differs for each gene, the formation and structure of the egg. These will be discussed in detail in a later paper.

These three genes appear to offer exceptional material for a study of the interaction of gene and cytoplasm in higher organisms. In each of the three, there is involved an extremely sensitive mechanism which can be lethal or non-

lethal depending upon either the genetic make-up of the female or the genes present in the zygote nucleus. It is especially fortunate that for at least one of the genes *(dor)* the time of action can be estimated very accurately, since it would seem to be dependent upon the entry of sperm into the egg, thus making possible the analysis of physical and chemical changes at the time when the initial action of the gene is felt.

In addition to the sterility effect, there are visible phenes associated with each of the genes; these may also be studied by developmental analysis. Since one of the genes *(dor)* affects eye pigmentation, the chemistry of which has been at least partially worked out, it may be possible to determine the stage in development of the viable homozygote when eye pigmentation deviates from normal, and how it does so.

The effects of the ring gland on the maintenance of the ovary and the deposition of yolk have been well-established (Thomsen 1940; Day 1943; Vogt 1940, 1941, 1943) and morphological and experimental studies of the ring-glands in these female-sterile mutants should prove of interest. Clancy and Beadle (1937) have made some studies of the effects of non-*fu* ring-glands on the development of the sterility characteristics of *fu*, by transplantation of the larval *fu* ovary into non-*fu* hosts, but no other studies have been made.

In conclusion, we can say that these three female-sterility genes because of their manifold effects during the course of development seem to offer promising material for further developmental and physiological studies.

Summary

1. The effects of a series of *r* alleles on embryonic development in crosses of mutant to mutant have been described.

2. Development in *r* embryos is normal until primary organogenesis is complete, but there is a complete cessation of differentiation between the thirteenth and sixteenth hours of development. Processes of differentiation which occur after this period are delayed, but occur normally.

3. Quantitative differences are observed in the patterns of damage of r^{39} and r^{41}. These reflect, in direction of degree of severity, quantitative differences which have been observed between the two in fertility, fecundity and production of abnormal eggs.

4. The presence of some males in crosses of *r* females to non-*r* males has been interpreted as evidence of a positive effect on development of supernumerary sperm in the egg cytoplasm.

5. The effects on development of *r* are compared with those of two other sex-linked genes—*fu* and *dor*—which exhibit a similar type of female-sterility.

6. In the light of increasing interest in the genetic potential of the cytoplasm the significance of nuclear-cytoplasmic interactions involved in the present study is commented upon.

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Kurze Mitteilung

Aus dem Max-Planck-Institut für Biologie, Abt. Melchers, Tübingen

EIN CYTOLOGISCHER BEITRAG ZUR „ENTMISCHUNGSTHEORIE" VERSCHIEDENER PLASTIDENSORTEN

Von

Roland Maly und Aloysius Wild

Mit 13 Textabbildungen

(Eingegangen am 14. Dezember 1955)

Erwin Baur (1908/09) erklärte das genetische Verhalten und vegetative Aufspalten der weißbunten Pelargonien durch die Annahme, daß in der befruchteten Eizelle ein Gemisch zweier genetisch verschiedener Plastidensorten vorliegt, und daß die später auftretenden Zellgruppen mit nur einer dieser Plastidensorten durch zufallsgemäße Entmischung bei den Zellteilungen entstehen. Renner (1934, 1936) hat sich nachdrücklich für diese Vorstellung eingesetzt; die kritischen Einwände von Correns u. a. sind bei Correns v. Wettstein (1937) zusammengestellt.

Diese Hypothese verlangt also Zellen mit zwei verschiedenen Plastidensorten, sog. Mischzellen, die solange vorhanden sein müssen als noch keine reinfarbig „weißen" oder „grünen" Gewebepartien größeren Ausmaßes abgespalten wurden.

Positive Angaben dieser Art wurden gemacht von Correns (1919), Funaoka (1924), Gregory (1915), Herbst (1935), Krumbholz (1925), Noack (1931, 1933, 1934), Schötz (1954), Woods und DuBuy (1951) und Yasui (1929). Schon Randolph (1922) und später Sturm (1938) nehmen gegenüber den Angaben von Mischzellen eine kritische Haltung ein. Entweder sind die vorliegenden Befunde nicht eindeutig, da sie die möglichen Fehlerquellen nicht beachten, oder sie stützen sich nur auf Farb- bzw. Größenunterschiede zwischen den Plastiden. Bei der öfter zitierten Angabe von Correns (1919) über Mischzellen bei *Capsella bursa pastoris* f. *albovariabilis* können Mischzellen im obigen Sinne gar nicht vorliegen; das *albovariabilis*-Merkmal wird durch ein labiles Gen weitergegeben.

Unser Material von *Antirrhinum majus* Sippe 50 umfaßt zur Zeit die als A—E bezeichneten Herkünfte des *status albomaculatus* (mit rein mütterlichem Erbgang). Die Buntheit in Mosaikform bleibt oft bis in die Infloreszenz erhalten, daneben kommen auch albotunicate und reinfarbige Äste vor.

Die abweichenden Plastidensorten der Herkünfte A—D sind cytomorphologisch nicht voneinander zu unterscheiden. Sie haben gegenüber der Normalform (s. Abb. 11) kleinere und je Flächeneinheit zahlreichere Grana (Abb. 1). Die Granastruktur kann sich so verfeinern, daß die Plastiden homogen erscheinen. Dabei tritt amöboide Formveränderlichkeit auf (Abb. 2). In schrumpfenden Plastiden erscheint die chlorophyllführende Substanz in größeren, ungeordneten Einheiten. Erst bei diesem Stadium setzt die eigentliche Ausbleichung der vorher hellgrünen Plastiden ein. Demgegenüber findet man oft innerhalb einer Plastide beträchtlich größere Grana neben den kleineren (Abb. 3). Im Extremfall kann bei gleichzeitiger Schrumpfung der Plastiden nurmehr ein „Granum" übrigbleiben (Abb. 4). Alle geschilderten Formen lassen sich als verschiedene Stufen

eines Degenerationsprozesses auffassen. In beiden Fällen kommt es schließlich zur Vakuolisierung; bei starker Beleuchtung tritt diese sehr früh auf.

Jeder Degenerationserscheinung eine mutative Abänderung zuzuschreiben wäre abwegig. Reinfarbig abweichende Blattsektoren, Blätter, Äste oder Mantel-

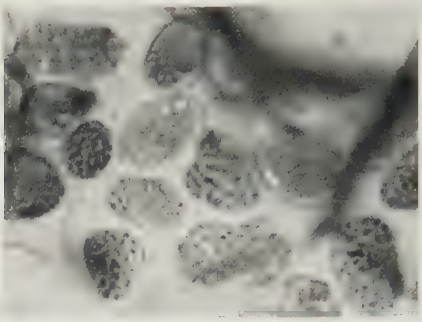

Abb. 1

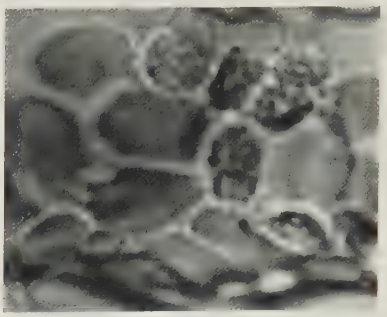

Abb. 2

Abb. 1[1]. Abweichender Plastidentyp mit ausgebildeter Granastruktur, vgl. Abb. 12 (normale Plastidensorte) (Herkunft B)

Abb. 2. „Übergangszelle" mit homogenen und geschrumpften Plastiden (Herkunft D)

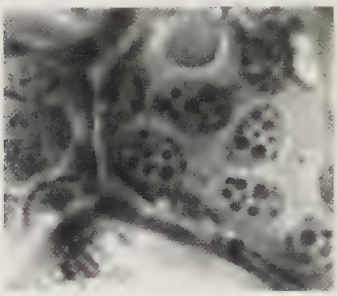

Abb. 3

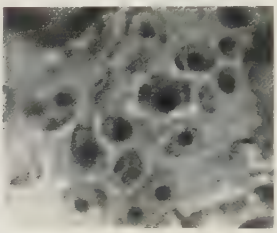

Abb. 4

Abb. 3. „Übergangszelle" mit unregelmäßig vergrößerten Grana neben kleinen Grana (Herkunft A)

Abb. 4. Geschrumpfte Plastiden mit stark vergröberten Grana (Herkunft D)

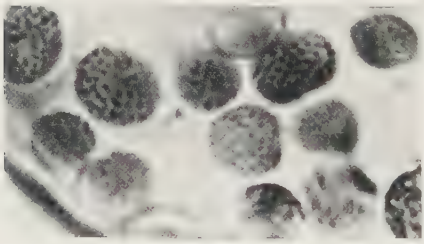

Abb. 5

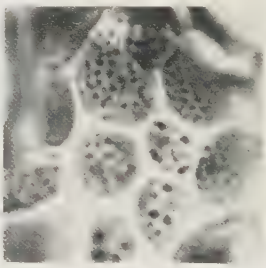

Abb. 6

Abb. 5. „Mischzelle", die abweichende Plastidensorte mit regelmäßiger Granastruktur (Herkunft C)

Abb. 6. „Mischzelle", die abweichende Plastidensorte mit unregelmäßig vergrößerten Grana (Herkunft A)

gewebe bei Albotunicaten zeigen stets alle möglichen Übergangsstadien. Niemals findet man etwa einen Sektor, der rein für nur eines der beschriebenen Stadien wäre — vom Endstadium abgesehen. Für das vorliegende Problem ist wesentlich, daß all die geschilderten Formen der einen abweichenden Plastidensorte in einer

[1] Sämtliche Aufnahmen sind von lebenden, unverletzten Zellen. Vergrößerung jeweils 1280mal, Optik: Leitz Fluorit 1/16 116mal, Ok 6mal, Miflex Leica-Aufsatz.

Zelle vorkommen können. Man erhält also *scheinbare* Mischzellen, die wir „Übergangszellen" nennen.

Die Existenz von echten Mischzellen läßt sich nur unter genauer Beachtung der Granastruktur der normalen Plastiden angeben. Selbst dann kann es

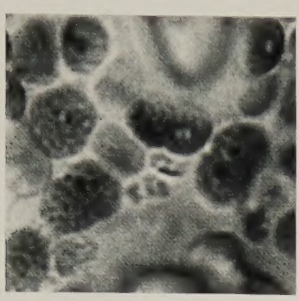

Abb. 7

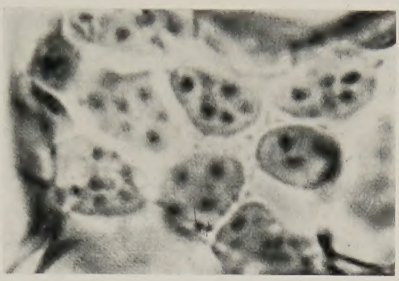

Abb. 8

Abb. 7. „Mischzelle" mit Übergang der abweichenden Plastidensorte von nahezu homogen zu geschrumpft (Herkunft D)

Abb. 8. Abweichende Plastidensorte von Herkunft E

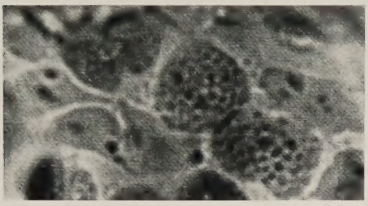

Abb. 9

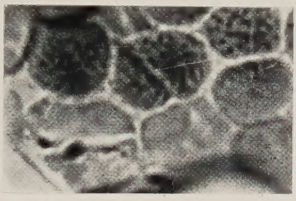

Abb. 10

Abb. 9 u. 10. „Mischzellen" der Herkunft E, Freilandmaterial. Die abweichende Plastidensorte leukoplastenähnlich mit amöboiden Fortsätzen

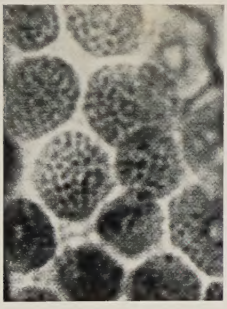

Abb. 11

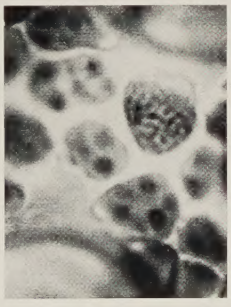

Abb. 12

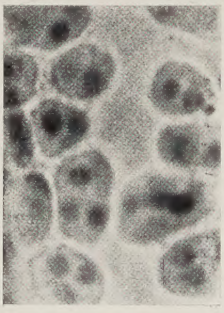

Abb. 13

Abb. 11. Zelle mit normalen Plastiden der Sippe 50 (Herkunft D)

Abb. 12. „Mischzelle" der Herkunft E

Abb. 13. Abweichende Plastidensorte der Herkunft E

sehr schwierig sein, wie Abb. 5 und 6 zeigen, solange die abweichende Plastidensorte noch eine gut ausgebildete Granastruktur besitzt. In einer Mischzelle kann die abweichende Plastidensorte auch in Übergängen vorliegen, wie Abb. 7 zeigt. Diese Art von Mischzelle stellt nicht etwa ein Gemisch von 3 Plastidensorten dar; wir stellen das im Hinblick auf die Angaben von Woods und DuBuy (1951) ausdrücklich fest, die "di-, tri- and tetrachondric cells" glauben gefunden zu haben.

Ein anderer Mutationsschritt liegt bei den abweichenden Plastiden der Herkunft E vor. Diese sind unter allen Entwicklungsbedingungen — untersucht von 1 cm langen Blättchen an — völlig klar von den Plastiden der Stammsippe zu unterscheiden. In den Plastiden finden sich wenige große Grana, die nur in 2—3 Schichten übereinanderliegen (Abb. 8, 13). Bei Freilandpflanzen oder im Gewächshaus bei zunehmendem Alter verringert sich bei diesen Plastiden der Durchmesser der Grana und sie erscheinen am Rand der Plastiden, die dann wie Leukoplasten mit eigentümlicher amöboider Formveränderlichkeit aussehen (s. die abweichende Plastidensorte in Abb. 9 und 10). Die mutierten Plastiden zeigen keine Stärke, auch dann nicht, wenn sie in Mischzellen vorliegen. Mischzellen sind hier besonders eindrucksvoll (s. Abb. 9, 10, 12). Sie erbringen einen klaren Beweis für die Baur-Rennersche Entmischungshypothese, der sich nicht wie bisher auf die nur unsicher entscheidbaren Farb- und Formdifferenzen, sondern auf die eindeutig von der Norm abweichende Granastruktur der mutierten Plastiden stützt.

Mischzellen sind an diesem Material — und zwar in allen Mischungsverhältnissen der beiden Plastidensorten zueinander — überall da zu finden, wo sie nach der Theorie zu erwarten sind. Es kann direkt an dem Scheckungsbild der Pflanze gezeigt werden, wo Mischzellen gefunden werden müssen.

Über die rein genetischen Untersuchungen zum Plastidenproblem bei *Antirrhinum* wird später ausführlich von Maly über die cytomorphologischen Befunde und über physiologische Versuche von Wild berichtet werden.

Der „Deutschen Forschungsgemeinschaft" danken wir für die Förderung unserer Arbeiten.

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Vorlesungen über Entwicklungsphysiologie

Von Professor Dr. Alfred Kühn, Tübingen. Mit 477 Textabbildungen. IX, 506 Seiten Gr.-8°. 1955. Ganzleinen DM 43.60

Aus den Besprechungen: Das vorliegende Buch Alfred Kühns ist ein Geschenk, für das ihm nicht nur der experimentierende Biologe, sondern auch alle dankbar sein müssen, die sich über den heutigen Stand der Entwicklungsphysiologie und ihrer Probleme zu orientieren wünschen. — Die unübersehbare Fülle der vorliegenden Teilergebnisse aus allen Organismenreichen kann nur durch einen Meister dieses Faches sinnvoll zusammengefaßt werden, der selbst — vielleicht als einer der letzten — noch in der Lage ist, so ziemlich das meiste davon aus eigener Anschauung zu kennen. In dieser Tatsache liegt der gar nicht hoch genug anzuschlagende Wert des Buches. — Sein Inhalt behandelt die Grundlagen der Entwicklungsphysiologie, angefangen von der spezifischen Struktur der Zellen und ihrer identischen Vermehrung über die Entwicklungsmöglichkeit der Zellen und die raum-zeitliche Ordnung ihrer Bedingungen innerhalb eines Organismus bis zur Wiederherstellung oder Veränderung des Erbgefüges und zum Beginn eines neuen typischen Entwicklungsgeschehens. Die Gliederung des ungeheuren Stoffes in 30 Vorlesungen scheint mir deshalb besonders glücklich, weil dadurch von vornherein der Anschein vermieden wird, als ob es heute schon eine allgemein gültige Theorie der Entwicklungserscheinungen gäbe. Statt dessen liegt jeder Vorlesung eine präzise formulierte Fragestellung zugrunde, aus der dann bestimmte Arbeitshypothesen entwickelt werden, welche oft genug an der Grenze unseres heutigen Wissens nur zu weiteren Fragen führen. Nur diese lockere Gliederung gestattet eine so ungewöhnlich große Auswahl von Beispielen aus der Tier- und Pflanzenwelt sowie aus dem Reiche der Protisten. Sie regen unsere Phantasie an, indem sie den Lernenden an Stelle von Spezialuntersuchungen die ganze Weite und Tiefe der Problematik des lebendigen Geschehens vor Augen führen. — Die reiche und schöne Bebilderung macht das Buch zugleich zum schönsten Bildatlas der Entwicklungsphysiologie, den wir besitzen. Auch dem Verleger gebührt dafür besonderer Dank.

Professor Goerttler, Freiburg i. Br., in „Berichte über die allgemeine und spezielle Pathologie"

... Die lebendige Darstellung vermeidet jede Schematisierung. Dem Leser wird Tatsachenmaterial in großer Fülle vorgelegt. Die Mannigfaltigkeit der deskriptiven und experimentellen Tatsachen ist gemeistert durch die induktive und systematisierende Herausarbeitung der typischen Vorgänge und der Funktionstypen. Nahezu 500 in einheitlicher Manier hergestellte Abbildungen erläutern den Text und zeigen in eindringlicher Weise, daß Wirkungen in der Entwicklung Gestaltungen sind. Die Liebe und Begeisterung des Autors haben ein faszinierendes Werk entstehen lassen, das jeden an der Biologie Interessierten durch Inhalt und Form, durch Auswahl und Formulierung, durch Herausarbeitung des Gesicherten und der Probleme fesseln muß und manchen verführen wird, sich diesem — wie der Verfasser sagt — charakteristischen Zweig der modernen Biologie zuzuwenden.

Professor Autrum, Würzburg, in „Berichte über die gesamte Physiologie"

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